ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH
1968-1969

NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE
VOL. I

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ANNUAL REPORT

July 1, 1968 through June 30, 1969

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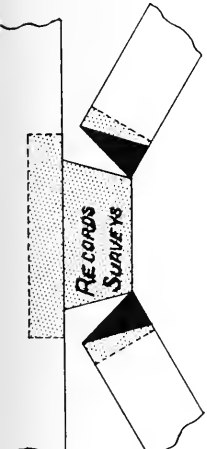

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SURGICAL
NEUROLOGY BR.
DIRECTOR'S REPORT
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MEDICAL
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ANNUAL REPORT

July 1, 1968 through June 30, 1969

National Institute of Neurological Diseases and Stroke
National Institutes of Health

The Director's Report

The year 1969 has been one of major transition. Several events have had a major impact on NINDS and its policies. The first of these was the resignation of Dr. Richard Masland as Director. The second event was the creation of the National Eye Institute which necessarily removes the major programs of research and training on vision and blindness from NINDB to the new Institute and resulted in the renaming of the remainder of NINDB as the National Institute of Neurological Diseases and Stroke.

The third important circumstance is that funding of the Institute is no longer increasing annually but actually decreased during FY 69, and further decreases are expected in FY 70. Furthermore, an attrition in personnel brought about by the prohibition of rehiring of 3 of every 10 employees leaving the Institute has resulted in serious understaffing in several areas.

In addition, funds available for foreign programs, and particularly foreign travel, have severely decreased.

The major impact of the events enumerated above has been and continues to be a careful examination and re-evaluation of all phases of the Institute's operations and policies with the aim of making optimal use of limited resources in the development of improved methods of prevention and treatment of neurological, sensory, and muscular diseases. Attempts are being made to identify those areas in which the state of basic knowledge is adequate to permit targeted programs to be directed toward specific disease problems. For example, the biological and technological base for the development of a device to supply additional sensory input to the blind through direct cortical stimulation (visual prosthesis) now appears to be adequate to warrant a major effort in the development of a useful device.

On the other hand, present basic knowledge has not yet reached the stage that will permit a major directed effort in the therapy of brain tumors, cerebrovascular disease, injuries to the central nervous system, communicative disorders, and many important fields. Strong programs of clinical and basic research must be continued in these areas in the hope that a major "breakthrough" will eventually occur in all of them and with the expectation that our knowledge and skill in

each of them will increase continually through a regular succession of small steps.

In other areas such as Parkinsonism the recent development of effective therapy (L-DOPA, Amantidine) and a better understanding of the role of the catecholamines in the brain makes accelerated programs of basic, clinical and epidemiological research in this area appear highly desirable even though the basic causes of the disease are not yet understood. Similarly, recent advances in our understanding of viruses and their relation to neurologic disease; [of development] such as measles virus and subacute sclerosing panencephalitis; and the transmission of (Creutzfeldt-Jakob) disease (Spongioform encephalopathy) from man to chimpanzees, indicate that expanded efforts in clinical as well as basic research are warranted.

The identification of genetically controlled enzyme defects as the causative agents in lipidoses also appears to warrant an expanded effort in applying this knowledge to the development of effective treatment. Such a treatment has already been found in the dietary management of children suffering from phenylketonuria.

Although much research is being done in the relation of viruses and auto-immune reactions in such diseases as multiple sclerosis and amyotrophic lateral sclerosis much more basic knowledge is needed before a significant improvement in treatment can be found.

A major undertaking has been the re-examination of the structure of various programs within the Institute, both by the administrative staff and by outside advisory groups. Some of the results of this continuing study are the following: After a thorough review by the Board of Scientific Counselors, the staff of NINDS and the Deputy Director for Science of NIH, the decision was made to move the Laboratory of Perinatal Physiology from Puerto Rico to the Washington area. There were several reasons for this decision--closer supervision and collaboration with other intramural laboratories; facilitation of the recruitment of highly qualified permanent staff members; elimination of duplicate administrative facilities; lack of relevance to the primatology programs at Cayo Santiago and La Parguera, and the heavy burden imposed upon the personnel of LPP in administering these programs. It is believed that the move will increase the efficiency of the laboratory as well as save money and eliminate unnecessary positions. We do plan, however, to retain and reprogram the primatology activities on the islands, and at the experimental compound facility Sabana Seca.

A study is being made of possible changes in the administrative structure of the Institute's direct operations in Bethesda with the aim of consolidating and simplifying administrative functions and procedures wherever possible.

As the Perinatal Research Branch moves its major emphasis from data gathering to data analysis a thorough re-evaluation of its structure and operation has

become necessary. Although two ad hoc review groups have examined the data gathering process in detail and the Perinatal Research Committee continues to visit and evaluate the operation of the individual contractors, until recently no outside review group has been charged with evaluating the internal operation of the Branch.

Accordingly, a site visit was made by the Perinatal Research Committee which has now submitted its report which is being studied carefully. In addition, a number of urgent problems are undergoing internal review. Some of these are the following: How to involve the individual contractors in the various studies on the data that have now been collected; how to acquaint the scientific community with the contents of the data file and to make it available to outside investigators; the organization of a unified and comprehensive data analysis plan and the assignment of priorities among the many possible studies.

Most of the facilities in Building 36 were completed in FY 69 and a major portion of IR and C&FR is now occupying them. In addition, space has been reserved for occupancy by the Laboratory of Biochemical Genetics of the National Heart Institute. The proximity of this highly talented and productive group is planned to permit the close collaboration with NINDS scientists, particularly those in the fields of neurophysiology, neurochemistry, neuroanatomy, and biophysics in studies on the role of biochemical genetics in the growth, development, and function of the nervous system. In addition, a neurophysiology laboratory being created in the National Institute of Child Health and Human Development will occupy space in Building 36 loaned by NINDS. This laboratory is being structured around personnel transferred from the Laboratory of Neurophysiology of NINDS and is expected to maintain close collaboration and share common facilities with our intramural scientists.

Although the use of NINDS space by these laboratories of other institutes will result in the decentralization of some of our own activities which must occupy space elsewhere, the concentration of activity in neurobiology of four institutes (NINDS, NIMH, NHI, and NICHD) in a single building should result in a unified interdisciplinary activity in neurobiology which can exert world leadership in the field and attract scientists of the highest caliber.

The Director's Laboratory

A small laboratory comprising 5 modules has been set up in Building 36 within the intramural program for the continuation of the research in which the Director has long been engaged. The object is the study of sensory input and coding in the nervous system and the study of the vertebrate retina as a model of central nervous tissue. The retina has unique advantages for this purpose in that it contains several synaptic layers in close proximity to the receptor cells and can be readily removed and maintained in isolation with its sensory input intact. Furthermore, the adequate stimulus is light which can be precisely controlled permitting highly quantitative

experiments.

Present personnel consists of the Director, Dr. Steven S. Shaw, a visitor from Scotland, and Mr. Ferenc Harosi, a graduate student in the biomedical engineering program at the Johns Hopkins University, who is doing a thesis problem at NIH.

Dr. Michael Wiederhold, a Staff Fellow, will arrive in September 1969.

Dr. Shaw is studying impulse generation in an invertebrate eye (barnacle) and is collaborating with the Director and the Scientific Director in summer investigations at the Marine Biological Laboratory, Woods Hole, Massachusetts, in research upon the horizontal cells of the eye of the dogfish. These cells are large and lend themselves well to electrophysiological studies. Their role in visual adaptations has been under study by the Director for several years.

Mr. Harosi is extending work previously undertaken by Dr. W. B. Marks, W. H. Dobelle, and E. F. MacNichol, Jr., at Johns Hopkins upon the visual pigments of the cones in the primate retina. These cells are responsible for the sensory coding of color. To date, technical limitations have prevented the study of the receptors in the fovea where the cones are densely packed, yet it is this region where visual activity is highest and information upon the spectral responses and relative numbers of cells having a given spectral response, is most needed.

The National Eye Institute

The creation of the NEI from NINDB has had a serious impact upon the workload in the Offices of the Director and of Extramural Programs.

It has been necessary for separate budgets to be prepared and defended for both institutes, to organize separate council meetings, and to divide the research and training projects formerly in NINDB into the two institutes. These activities together with contract renegotiation and other additional workloads imposed by personnel reduction and other problems related to financial stringency have imposed almost intolerable burdens upon the staff involved in doing the extra work. The personnel have done this work extremely well and deserve the highest commendation for the long hours and hard work that have been required.

It is anticipated that the major effort required in separating the NEI has been completed though continued assistance must be provided until the NEI secures an adequate administrative staff. The separation of intramural research, which should be largely accomplished in FY 70, does not appear to present such serious problems since the Ophthalmology Branch is a compact and well defined organization and additional space and personnel for expansion of the program will probably become available. It may be desirable to jointly administer some programs for the foreseeable future since they are not large enough to warrant duplicate administration.

The Office of Program Analysis

The Office of Program Analysis is responsible for: providing information of all NINDS activities and the relationship of other government and non-government agencies to the overall objectives and goals of the NINDS; serving as a focal point for certain NINDS activities such as foreign research, manpower problems, liaison with other institutes, government agencies, committees etc. so that questions concerning research in neurology throughout the world may be quickly and accurately answered; developing and assisting in the development of plans for the achievement of the major NINDS goals and objectives.

File of Scientific Data

To accomplish its objectives, all of the projects supported by the NINDS (Intramural, Extramural and Collaborative and Field) are indexed and the terms punched on keydex cards. There are approximately 1500 terms allowing for coordinated searches in considerable depth.

In order to facilitate the retrieval of information on the Extramural Program, a copy of the abstract (in the form of Notice of Research Projects received from the Science Information Exchange) and a copy of the descriptor sheets (received from DRG) are arranged in notebooks according to disease category. These are constantly updated to aid in identifying research trends within given areas.

During this past year, a total of 26,502 items, more than 2,200 items per month, were handled for consideration for inclusion in the scientific information file. Some 8,650 were either reprints, abstracts, or manuscripts which were the results of research. These are all filed with the data kept on each project so a record might be available which is as complete as possible.

Plans are underway at present to convert our system of data storage to microfiche. This will allow us to store more material more efficiently and economically.

The Secretary's Committee on Mental Retardation has published a two-part study entitled Mental Retardation Grants. Part II includes those grants awarded by several agencies in Research and Demonstration. The Office of Program Analysis supplied the necessary information to the Committee and developed the system of classification used in the publication.

Reports, Publications and Workshops

The cerebrovascular disease alerting service, providing monthly bibliographical listings and abstracts of material from about 145 journals through a contract with the Mayo Foundation of Rochester, continues to be a worthwhile service. Many special reports have been generated throughout the year in response to various Institute needs. The following

are noteworthy: Federally Supported Agencies with Programs in Neurological and Sensory Disorders - a detailed description of the programs of government agencies other than NIH which have significant programs in the field of neurological and sensory diseases; International Activities of NINDS - a comprehensive review of NINDS - sponsored foreign research through both the Extramural Programs and the Foreign Credit Currency Awards; Current Drug and Drug-Related Research in Neurological and Sensory Diseases - an analysis of NINDS sponsored and non-NINDS sponsored research on drugs in neurosensory disorders; NINDS Research Programs in Vision (FY 68) - a comprehensive picture of the Institute's supported research in the field of vision; Summary NINDS Dyslexia-Related Grants - a review of NINDS - supported research in dyslexia for FY 68; Inherited Disorders with Proven Metabolic Defect - a summary of inherited disorders that have been shown to have a proven metabolic defect (of the 72 disorders listed, the defect in 9 has been located by NINDS grantees).

A supplement to the report Progress in Spinal Cord Injury Research FY 1967 is in preparation as well as a supplement to Research Relating to Tumors of the Central Nervous System.

A state-of-the-art report on Multiple Sclerosis, Amyotrophic Lateral Sclerosis, and Experimental Allergic Encephalomyelitis is near completion. A report on current research trends in infectious diseases of the nervous system is in preparation. A review on cerebral palsy will be written during the coming year.

With the cooperation of MEDLARS, the Office of Program Analysis is establishing a continuing bibliography of case reports and reviews of neurological and sensory diseases. The objective is to determine the relative rarity or frequency of unusual diseases and rare complications.

In March 1969, the second experimental edition of Neurological Research supported by NINDS appeared. This contains data on ongoing intramural, extramural and collaborative and field research projects as well as data on research contracts supported by the Institute. Each entry contains a citation, abstract, list of descriptors, project number, investigators and location of work. The entries are arranged according to disease category. A Subject Index, Principal Investigator Index, Applicant Institution Index and Project Number Index are included to aid the user.

On December 5, 1968, the Office of Program Analysis sponsored an intracranial pressure workshop, bringing together many clinicians and medical engineers to discuss the state of the art of intracranial monitoring.

Epilepsy Information - Storage and Retrieval

The Office of Program Analysis in cooperation with the Epilepsy Section of Collaborative and Field Research made notable progress toward its goal of a computer-based secondary information system for epilepsy.

With the introduction in May 1969 of the Data Central, an on-line interactive computer controlled information storage and retrieval system, it became possible for the first time to search free text by computer across the full range of the world's current epilepsy information. The Epilepsy Data File "EPIL" consists of machine-searchable records containing the title, author's names, complete bibliographic citations, indexing terms, and complete information abstract for each article abstracted in Epilepsy Abstracts (EA). The new pilot service is a product of a system initiated by the Institute in 1967 for unified computer processing of abstracts for EA.

A demonstration-workshop of the system was held on May 8, 9, 1969 for the Public Health Service Advisory Committee on the Epilepsies.

Information on the 1968 EA literature forms the beginning of the Institute's computerized data base. In addition to producing EA, the format allows for at least four main uses of the master file: computer controlled production of year end indexes for the respective journal's citations; production of special-combined selective or cumulative printed indexes or bibliographies; information retrieval project and tape products.

Neurological Information Network

The Neurological Information Network has had a steady productive growth of service to the point where it can be said that its activities have affected almost all of its potential audiences at least once. The users have expressed pronounced enthusiasm and satisfaction with the Network operation and its services. The current awareness bulletins and state-of-the-art reports continue to receive national and international acceptance. The Network has become highly visible in countries outside the United States where such information services are far less common.

The Brain Information Service (BIS) through the workshop mechanisms has produced three important reviews during the past year. Continuing service was rendered to the scientific community through the transmittal of copies of publications, bibliographies prepared on special request, continuing bibliographies and reproduction of material and tables of contents. The BIS continued to strengthen its program for output with its Index to Current EEG Literature published quarterly, the bi-weekly Current Awareness Service Sleep Bulletin and the bi-weekly Neuroendocrine Bulletin.

Equally impressive has been the progress at the Parkinson Information Center (PIC) where the bi-weekly alerting bibliography, Parkinson's Disease and Related Disorders: Citations from the Literature and retrospective literature searches continue to be in great demand. There continue to be about 250 requests monthly for copies of special bibliographies. A second edition of the review Parkinson's Disease Present Status and Research Trends which will deal with progress and trends in 1965 - 1968 is now in press. The PIC function of indexing the neurological literature for MEDLARS and announcements of Index Medicus

continues to be highly effective. At the 1968 annual national meeting of the American Society for Information Science (ASIS), one of the highlights of the convention exhibit was System Development Corporation's (SDC) demonstration of its on-line Retrieval of Bibliographic Text using the PIC data base.

The Information Center for Hearing, Speech and Disorders of Human Communication has had an active year. An authoritative State-of-the-Art Report on the Acoustic Neurinoma is completed and will be published in Acta Oto-laryngologica in 1969. The State-of-the-Art Report on Viral Infections and the Neonatal Nervous System is in preparation and will be completed in 1969. The first issue of the current awareness publication, Communication Disorders was distributed in May 1968, and now has a mailing list of over 800 from 46 states and 25 foreign countries. The Center also produced a guide to sources of information in this area entitled, Information Sources in Hearing, Speech and Communication Disorders. A variety of other bibliographic-related activities has been undertaken by the Center.

The Vision Information Center (VIC) has made a great contribution to the field of biomedical communication with its development of an on-line bibliographic information retrieval system and the computer-assisted instruction program. As a result of the efforts of the VIC, 2 major journals in ophthalmology have adopted the policy of publishing descriptors and an abstract with each article to facilitate indexing, abstracting and retrieval by information centers. VIC has supplied bibliographic support and indexing for the annual reviews published in the Archives of Ophthalmology and has begun a cooperative indexing project with the National Library of Medicine.

A subcommittee for glossary development and indexing has been meeting to assist the Network and provide guidelines to each center for the development of specialized thesauri (micro-thesauri) in the neurosensory sciences. This development promises to materialize as a major contribution to the field of information science.

In cooperation with the Information Office, an exhibit describing the need for our Neurological Information Network and how it works was prepared to assist researchers, practitioners and educators. This was shown at the American Academy of Ophthalmology and Otolaryngology in October 1968 and at the American Laryngological, Rhinological and Otological Society in March 1969. It is scheduled for several showings in the coming year.

State University of New York (SUNY) Project

The SUNY Project, a cooperative effort involving SUNY, the Parkinson Information Center (PIC), the National Library of Medicine, the Vision Information Center (VIC), and the NINDS has provided the NINDS Network with a facility to interact with the Biomedical Communication Network (BCN). With the establishment of PIC as one node in an on-line station in BCN, the Neurological Information Network will be able to provide valuable experimental data concerning the efficacy of different methods

of on-line searching using different sets of thesauri. The PIC terminal coupled with the Harvard terminal to the BCN provides the NINDS Network with the first stage of a linkage between all of its neurological information centers adding to, and searching a collective data base.

Research and Training Manpower Section

A manpower file, containing 2,719 names has been set up making it possible to identify physicians and scientists by name, specialty, certification, geographical location, position and scientific interests. Other scientists working in disciplines of interest to NINDS but supported by different agencies will eventually be added. Review and updating of the manpower survey is a major project of this section. Also in preparation is a report on careers pursued by physicians and scientists who received NINDS support for part of their training during the years 1965 - 1968. The report will include tabulation on age, sex, academic degrees, current type of practice and address, specialty, training and experience. It will also include listings of institutions in this country and abroad where the trainees obtained their professional degrees and the specific disciplines on their training.

The Office of Public Information

The major mission of the Office of Public Information, NINDS, is to inform the public of the latest neurological and sensory research advances. Millions of people in this country suffer from such long-term neurological and sensory disorders as cerebral palsy, muscular dystrophy, multiple sclerosis, epilepsy, stroke, parkinsonism, head injury and deafness. Since many of these diseases or conditions are both chronic and progressive, it is especially important to provide those who suffer as well as their families and friends with the latest information on advances in treatment and medical research.

In response to this growing demand, the Office of Public Information has continued to rapidly expand its comprehensive information and public relations program. The greatest single area of growth has been in the distribution of Institute publications to the professional and lay communities. By early 1969, the number of individual copies of pamphlets distributed by the Information Office exceeded one million. Efforts to reach more people engaged or interested in the health professions are now showing results. These people include physicians and scientists, educators, members of Congress, voluntary health agencies and foundations, State and local health officials, and private citizens.

Publications

During the past fiscal year, more than 380,000 copies of various types of brochures were distributed through the publications program. The Office of Public Information has been very successful this past year in reaching a greater number of people in its professional audiences. For example, thousands of publications were supplied to nurses and nursing educators for use by their patients and students. A pamphlet on the medical problem of dizziness was made available to nearly 6,000 otolaryngologists for distribution to their patients.

A number of new publications were printed during the year, including additions to three continuing series. In the Hope through Research series written for the lay public, "Spinal Cord Injury" (the 16th pamphlet in this series) was published, while nine other HTR pamphlets were revised. Research Profiles (pamphlets on research advances for scientists and well-informed laymen) were combined in 1969 into one illustrated 41-page booklet.

Four new monographs were added to the NINDS monograph series. These monographs include: "Refractive Anomalies of the Eye"; "The Clinical Neurophysiology of Epilepsy: A Survey of Current Research"; "Public Health Aspects of Hearing, Language, and Speech Disorders"; and "Drug Trials for Headache, Principles and Methods."

A new 10,000 word illustrated brochure is now in press which stresses early testing of hearing, speech, and language disorders in children. The booklet

provides a chronological description of how children talk and describes the appropriate methods and games for language stimulation of different age levels. A second brochure was written on the Institute programs and has now been submitted for final clearance. This new Institute brochure should provide greater understanding of the Institute's total research program.

Other new publications include fact sheets and statements on L-DOPA, a leaflet for tourists and other visitors to NINDS monkey colonies in Puerto Rico in both Spanish and English, and a pamphlet on the Collaborative Perinatal Research Project compiled from a series of articles written for Gannett Newspapers. The Information Office also published a leaflet, "The Child with a Reading Disorder: A Fact Sheet for Parents," for the Secretary's (HEW) National Advisory Committee on Dyslexia and Related Reading Disorders. A survey article on maternal rubella and its effects, written by an Information Office staff member, was reprinted for use in answering the many letters on this subject.

Working closely with medical schools and grantee institutions, the Information Office is making progress toward providing closer liaison between the Institute and these organizations. For instance, "NINDS Link," a newsletter issued several times a year, was designed to bring information about the Institute's extramural programs to Institute grantees, Institute Advisory Council members, Institute committee members, university budget officers, Institute personnel and others associated with NINDS grant activities.

Six issues of the "NINDS Review" were written and published for distribution to voluntary health agencies and NANDS Council Members. A new check list with about 45 titles suitable for scientific and lay publics was widely distributed.

The Office of Public Information continues to provide editorial, production, and distribution services for Epilepsy Abstracts, a monthly journal sponsored by the Institute's Section on Epilepsy. The publication is now sent to approximately 3,100 U. S. and foreign scientists, teachers, and others with a working interest in the field.

Public Inquiries

The number of requests to the Office of Public Information for information on the neurological and sensory disorders has nearly doubled during the past year. Short radio messages, both at the Institute and NIH level, have greatly increased demand for information on neurological problems.

In addition to requests for specific publications, thousands of written public inquiries were received by the Office of Public Information. This year, a new drug (L-DOPA) used in the treatment of Parkinson's disease was the subject of hundreds of letters and phone calls. More than 2,100 required individually prepared letters in answer to specific questions. Many of these letters were in response to inquiries from Congressional offices. In addition, the Information Office was able to answer a large percentage of incoming mail through the use of printed material. Also, more than 1,500 inquiries were answered by phone.

Press and Periodicals

About 140 reporters, feature writers, editors, and broadcasters requested Institute information. The Office prepared and distributed 8 press releases and announcements, and held a number of press briefings. Ten articles were written for the NIH Research for Health series and sent to daily and weekly newspapers. Features were written on epilepsy, spinal cord injury, muscular dystrophy, retinal detachment, diabetic retinopathy, mental retardation, hearing loss, the cornea, and glaucoma. A number of stories also were written for the agency house organ, the "NIH Record".

Interviews were arranged with the NINDS Director or other scientists for articles and stories used in the scientific and lay press. Magazines, medical journals, and newspapers publishing stories included: Look, Changing Times, Electronics Magazine, Popular Science Monthly, Time, Today's Health, Medical Tribune, U.S. Medicine, Wall Street Journal, Washington Post, and Good Housekeeping.

Press Conferences and Meetings

In the fiscal year 1969, the Neurology Institute was the source of at least three major events of nationwide interest to the press and other media. First, the creation of the National Eye Institute stirred widespread interest and was the source of many stories by those interested in vision programs and research. There was also growing interest in a new drug, L-DOPA, developed with Institute support, and found effective in treating parkinsonism. In another research area, the Institute has provided evidence that a virus (measles) is involved in at least one neurological disorder. On March 10, 1969, the NINDS Information Office held a briefing for the medical press to announce the latest findings about the rare nuerological disease, subacute sclerosing panencephalitis (SSPE). (This finding opens up a number of research leads in the possible viral etiology of other chronic diseases.)

Press briefings and interviews with reporters were also conducted to announce other important scientific findings or developments. The Information Office arranged press interviews to announce the transmission of Jacob-Creutzfeldt disease to a chimpanzee. A press briefing was held on May 21, to announce conclusions derived from a meeting on Immunological Responses to Perinatal Infections. The Information Office also assisted members of the press attending the International Conference of Rubella Immunizations and the International Conference on the Late Effects of Head Injury.

Exhibits

At least three program exhibits are kept current and shown at national meetings of professional societies and lay organizations. This past year the Information Office designed three special exhibits. A scientific exhibit on multiple sclerosis research was produced cooperatively with the National Multiple Sclerosis Society. Another exhibit was designed to describe the function of the Institute's Neurological Information Network. A special publication exhibit is being used to obtain more visibility for

NINDS publications. This exhibit is displayed by medical societies, voluntary agencies, and schools.

The Office arranged and manned exhibits at such national meetings as American Academy of Neurology, American Academy of Ophthalmology and Otolaryngology, American Speech and Hearing Association, National Multiple Sclerosis Society, United Cerebral Palsy Association, and the South Carolina Health Science Fair.

TV and Radio

A new record of 20 radio spots was made by NINDS and distributed to 5,000 radio stations. The record stresses the need for research and cooperation with voluntary agencies. These were recorded by four Senators and 10 Hollywood personalities including Raymond Burr, Fess Parker, James Drury, and Agnes Moorhead. The Senators were Javits, Dirksen, Hill, and Byrd of West Virginia.

Arrangements have been made to produce 13 tapes in 11-minute segments to appear for 13 weeks on the special WTTG TV program, Panorama. Later, it is planned to offer these tapes to other TV stations around the country.

Reports and Special Documents

The Information Office helped prepare various Congressional materials required by the Director during the appropriations hearings. In addition to materials for the opening statement, these included eight special reports, a document "Highlights of Research" summarizing 10 areas, and other materials for NINDS as well as NEI testimony.

A number of other special reports were written, including a special information report to the Senate summarizing efforts to gain a wider audience for Institute programs.

During the year, 47 journals were scanned on a weekly or monthly basis, and 35 research weeklies, as well as program development items, were prepared for the Director, NIH, and the Assistant Secretary for Health and Scientific Affairs using grantee or Institute scientists' findings.

Arrangements were completed for many Institute lectures, including the preparation and distribution of flyers.

Speeches and Special Statements

Nine speeches were written for delivery be the NINDS Director, Assistant Director, and the Surgeon General. On several occasions, suggested messages or proclamations from President Johnson, and later from President Nixon, requested by leading voluntary organizations marking special events, were written.

Films

The Information Office is expanding its effort to stress the need for early recognition of neurological and sensory disorders in children, in order that treatment can be instituted, language can develop normally, and the children can fit into regular education classes. A color motion picture is in production which will help parents and teachers recognize minimal brain dysfunction before children go to school. The work on this picture has been completed except for minor script changes. Recently a number of speech and language experts serving as advisors saw a working print and all agreed that the final production is excellent. At least 30 prints of this movie will be made available for distribution to interested groups through the National Medical Audiovisual Center.

Cooperation with Voluntary Agencies and Foundations

The NINDS Office of Public Information worked closely with the National Committee for Research in Neurological Disorders and its many members--some 20 national voluntary health agencies and 10 professional societies in the neurological and sensory area. Examples of such cooperation included the production of cooperative scientific exhibits, assistance with the distribution of films, cooperation in the production and distribution of pamphlets, and assistance with national and regional meetings of various voluntary agencies. These included special projects this year with The Deafness Research Foundation, Epilepsy Foundation of America, Muscular Dystrophy Associations of America, Inc., National Association for Retarded Children, National Multiple Sclerosis Society, National Paraplegia Foundation, National Easter Seal Society for Crippled Children and Adults, Paralyzed Veterans of America, Inc., and United Cerebral Palsy Associations, Inc. Included also were special services to American Association on Mental Deficiency, American Association of Ophthalmology, American Orthopsychiatric Association, Council for Exceptional Children, and National League for Nursing.

Inter-American Activities

Goals

The goals of the Inter-American activities in Puerto Rico continue to be: 1) investigate the research and training potential in Puerto Rico and Latin America as they related to NINDS; 2) contribute to the growing NINDS programs in neurological science in Puerto Rico; 3) advance, integrate, and coordinate NINDS activities in Puerto Rico as they relate to NINDS activities in the U. S. mainland and other sectors of the Americas; 4) in a staff capacity, help develop creative and meaningful liaisons of NINDS programs in Puerto Rico with their counterparts in the Americas, and 5) thus aid in the development in Puerto Rico of a creative setting and ambiance for a true Inter-American interchange of neurosensory information, ideas and developments. In this way, the NINDS investment in Puerto Rican neurological science can be reinforced by having the Puerto Rican establishment in neurological science function as an important connecting link of scientific exchange between the neurological communities of the Americas.

For NINDS programs in Puerto Rico to constructively fulfill their mission, Puerto Rican neurological science should have a creative identity as is envisaged in its growth as a central focal point for an interchange of knowledge in the neurological sciences between North and Latin America.

To aid in the achievement of these goals as they work to the benefit of NINDS's involvement, is the central purpose of the Inter-American activities. These aims have not and do not in any way relate to the solicitation or procurement of NINDS grant awards.

Methods

Progress toward the attaining of these goals has been made by: 1) evaluation of NINDS neurosensory programs in Puerto Rico through interviews with Puerto Rican officials and department heads, consultations, seminars and lectures at the Puerto Rico School of Medicine and the Medical Center; 2) visits to all academic and research medical facilities in Puerto Rico and in Latin America when consonant with NINDS policy; 3) extensive correspondence with leading neurologists and neurological scientists from Latin America; 4) offtime participation in the activities in the Puerto Rico academic and voluntary agencies program that are dedicated to neurosensory disorders and preserves contact with the Perinatal Laboratory in an advisory capacity.

The Chief of Inter-American activities has a background knowledge and experience for participation in such activities. He is, for example, Professor of Clinical Neurology at the School of Medicine, University of Puerto Rico; an active member of the Puerto Rico Medical Association; member of the Advisory Board of the Section of Psychiatry, Neurology and Neurosurgery of the Puerto Rico Medical Association. He is the founder of the Puerto Rico Academy of Neurology and Alternate Delegate from Puerto Rico to the world congresses of neurology; Honorary President of the Second Pan American Congress of Neurology; honorary member of all Latin American neurological

societies and recipient of the highest government order of the country of Peru (Comendador de la Orden "El Sol del Peru"). He has command of both Spanish and French. He is on the Medical and Research Advisory Boards of the local voluntary societies dedicated to epilepsy and muscular dystrophy, as well as the program for crippled children and adults.

Activities in Fiscal Year 1969

At a meeting held in San Juan, the Puerto Rico Chapter of the Muscular Dystrophy Associations of America was constituted under the provisional chairmanship of the District Directors (Southern District). Since the disease is prevalent in Puerto Rico, and because of his association with muscular dystrophy and his knowledge of muscle diseases, the Chief of Inter-American Activities serves as Scientific Advisor of the newly established Puerto Rico Chapter.

During FY 1969, your reporter attended the meetings of the Advisory Board of the National Multiple Sclerosis Society and the Executive Sessions of the American Neurological Association. He also visited the National Library of Medicine for the procurement of necessary references for the program in Puerto Rico.

Met with officers of the Muscular Dystrophy Associations of America in New York with respect to development and coordination of programs related to NINDS activities in Puerto Rico, and for forming a greater liaison with the Puerto Rico Chapter and the University of Puerto Rico School of Medicine.

Consulted with the new Director of NINDS relative to coordination of NINDS programs. Made arrangements with the Chancellor of the Medical Sciences Campus and the Dean of the University of Puerto Rico School of Medicine and other leading medical authorities for the visit of the new Director of NINDS to Puerto Rico, to discuss with them the NINDS activities.

Participated in the annual sessions of the American Academy for Cerebral Palsy in Miami and conferred with District and Regional Directors of the Muscular Dystrophy Associations of America.

At the meeting of the Board of Scientific Counsellors, your reporter recommended the establishment of a Committee on Liaison for the coordination of activities of NINDS, the formulation of a program with the major communication points such as the University of Puerto Rico School of Medicine, the Department of Health, the Puerto Rico Medical Association and local voluntary health agencies. The implementation of such programs contribute to the public image of NINDS activities in Puerto Rico.

Your reporter has been interested in following reports on the effectiveness of L-Dopa in the treatment of Parkinson's disease. At the request of the Director of NINDS, visited the Parkinson Foundation and the Parkinson Research and Rehabilitation Institute in Miami, on an exploratory basis for the possibility of providing connecting links between the Miami program and a project at the University of Puerto Rico which leads to other parts of Latin America.

During FY 1969, the Office of the Director of Inter-American Activities was given the assignment of collecting papers and presentations from the Second Pan American Congress of Neurology (October, 1967), which was organized by your reporter in conjunction with the University of Puerto Rico, and supported in part by a grant from NINDS; these papers and presentations will be published in a volume of proceedings in Spanish and English, of the Congress. This project is nearly completed, the galley proofs are now being corrected and the volume will probably appear sometime in October 1969. The printing of the volume is being financed by the Department of State in Puerto Rico. The printers are the Talleres de Artes Graficas of the Department of Education.

Office of Biometry

The Office of Biometry, NINDS, is a branch within the Office of the Director, NINDS, and operates under the guidance of the Assistant Director of the Institute.

The Office of Biometry consists of the Office of the Chief and of four Sections, each with a specific mission. The primary objectives of the organization are to provide all arms of NINDS with a central source on which to draw for service in statistics, mathematics, data processing, and computer technology. A secondary aim is to provide consultation to other organizations undertaking researches in the area of NINDS interest.

The following is a brief description of projects in which the Office of Biometry provided consultation and computational service in the design, analysis, and evaluation of researches and experiments by the clinical, basic, and collaborative-research scientists of NINDS. The listings are by the unit of NINDS to which service was given, or by the academic institution to which consultation was provided.

OFFICE OF THE DIRECTOR, NINDS

1. In the interest of the Institute's effort to advance research into reading disorders, the Office of Biometry provided consultation to several scientists studying the problem of dyslexia. These include Dr. Barron of the DHEW National Advisory Committee on Dyslexia and Reading Disorders, who is designing a program testing diagnostic and remedial procedures to be undertaken at the West Texas Rehabilitation Center, Abilene, Texas; Dr. Mark of Johns Hopkins University, who has developed a diagnostic procedure; Dr. Ozer, George Washington University, who is testing a diagnostic method; and Dr. Waites of the Shrine Hospital for Crippled Children, Dallas, Texas, who is developing a program in which to collect information on children referred to the Reading Disability Clinic.

2. Provided consultation to scientists working in ophthalmology and related fields, as part of the Institute's program to strengthen epidemiological research on disorders of the eye. Studies involved include an evaluation of eye disorders in animals at several university hospitals, an eye pathology research program at several university medical centers, and a study of factors related to the success of corneal transplants, undertaken at San Francisco General Hospital, for which the Office of Biometry is completing a discriminant analysis.

3. Participated in the Institute's program toward the evaluation of the efficacy of L-DOPA, a promising anti-Parkinsonism drug, and its toxicity. The Office of Biometry is a current participant in the Institute's effort directed toward the design of a study to measure long-term effects of the drug.

4. The Office of Biometry maintains the Model Reporting Area (MRA) for Blindness Statistics, and coordinates the activities of the 18 States currently involved in the program. It is the central source for the complete statistical and data-processing function of the program, the objectives of which are to develop uniform data on visual acuity and blinding condition, along with data on the characteristics and attributes of the blind.

5. The Office of Biometry provides the Project Officer for the Perinatal Project maintained under P.L. 480 funds in Warsaw, Poland. OB has participated in designing a series of studies to be carried out over the next three years of this project.

6. Provided service in production of data for review by Perinatal Statistical Ad Hoc Committee appointed by NINDS to make a thorough review of the data of the Collaborative Project and had representatives at all meetings of Committee to interpret data, meet Committee's requests for material for evaluation, etc. Reviewed draft of final report for accuracy of tabulations.

7. Developed information for the Perinatal Research Committee, which serves as an NINDS Council for the Collaborative Project, as the Committee requested it. An example of this information is the relating of findings of abnormality for a given child at nursery, one-year, four-year, and seven-year examinations.

8. Applied the KWIK system as a means by which to automate the reprint libraries and files of the senior staff of the Institute.

EXTRAMURAL PROGRAMS

1. Provided consultation to the Associate Director for Extramural Programs in preparation for his analyses of the Research Grants Program and the Training Grants Program.

2. Provided consultation in certain research programs in which the Institute has an involvement -- as, for example, the study of diabetic retinopathy.

COLLABORATIVE AND FIELD RESEARCH

Epidemiology Branch

1. Tabulated and analyzed data developed in study of multiple sclerosis and selected antigens.

2. Provided consultation for study of long-term effects of surgery on patients with unilateral Parkinson's disease.

3. Provided consultation for study of natural history of patients with unilateral Parkinson's disease.

4. Assisted on design of study of dystonia Musculorum Deformans.

5. Assisted on design of Neuroma study.

6. Analyzed data in study of relationship of measles and subsequent neurological symptoms.

7. Tabulated data for study of diabetic mothers on Guam.

8. Provided consultation and statistical assistance in design of experiment for study of Kuru and non-Kuru chimpanzee cerebrospinal fluid after injection of gamma globulin. Analyzed data presently available for differences between Kuru and non-Kuru chimpanzee's cerebrospinal fluid, using analyses of variance and t-tests.

OPHTHALMOLOGY SECTION

1. Provided consultation for a genetic study of the level of sensitivity in the eye to topical steroid. Assistance supplied for an intra-twin correlation analysis of the intraocular pressure response of monozygous twins and dizygous twins to topical steroid, and for comparisons between these two groups and interpretation of results.

EPILEPSY SECTION

1. A member of the Office of Biometry is on Committee on Anti-Convulsant Drugs.

2. Participated in study to evaluate the clinical efficacy of Zarontin, an anti-convulsant, as an agent for the treatment of Petit Mal. Contributed to formulation of hypotheses, experimental design, and quality-control procedures, and specified statistical analyses of results.

3. Developed the experimental design for the clinical trial, Phase I, of Albutoin, a new anti-convulsant drug. Data from this study, undertaken at the State Hospital, New Castle, Indiana, will be processed and analyzed by the Office of Biometry. A plan for allocation of patients in Phase II is under development.

4. Participated in design of study for evaluation of several anti-convulsants, taken singly and in combination, to establish dose-response curves. Designed data-abstraction forms, established data-flow system, and developed quality-control methodology. Analysis of data and interpretation of results will be undertaken by the Office of Biometry.

5. Provided the investigator for study of association between delayed skeletal maturation and epilepsy.

6. Developed data-processing methodology for Collaborative Study of Epilepsy. Preparing currently, in conjunction with collaborators, monograph on findings of Collaborative Study of Epilepsy.

7. Prepared specifications for an information retrieval system for the production of reprint files for the Epilepsy program and for the Head Injury program.

Perinatal Research Branch

1. Handled all aspects of the extensive data-processing requirements of the Perinatal Collaborative Project, and provided staffing for the unit attached to the Perinatal Research Branch that performs this function.

2. A member of the staff of the Office of Biometry is on the Data Analysis Board, a PRB body that reviews study requests for approval.

3. Developed and staffed program to provide for the quality-control of 3-, 4-, and 7-year examinations.

4. Participated in study of association between EEG characteristics and high-risk seizure patients selected from 7-year-old Project patients, developing data-collection protocols, holding workshops, instituting quality-control procedures, and specifying statistical analyses.

5. Designed an experiment and developed a corresponding statistical analysis suitable for estimating the reproducibility of serological tests. A probability model was developed to describe the experimental error structure of these tests, as was a maximum likelihood iterative procedure for the statistical analysis of data.

6. Designed for the Behavioral Sciences Section, and analyzed the results of, a study to evaluate the 8-month Bayley Scales as a predictor of the IQ of a child at a later (4-year) stage in his development.

7. Participated in the design, tabulation, and analysis of a study relating the effects of maternal asthma to the outcomes of pregnancy. Co-authored (with researchers from New York Medical College and University of Buffalo) a paper currently in press (Am.J. Ob. & Gyn.).

8. Designed a study, using a discriminant analysis, to attempt the identification of high-risk pregnancies at time of first observation by an obstetrician of women not overtly at risk. Paper presented at most recent meeting of APHA.

9. Consultation and statistical assistance were given for a study of the possible associations between neonatal sepsis and some predisposing factors resulting from obstetrical complications.

10. A statistical analysis was completed for a gamma-immunoglobulin Study. Two papers arising from this study -- determining gamma-globulin level by precipitin ring method, and gamma-globulin M level and rubella -- have been accepted for publication by the Journal of Pediatrics.

11. Participated with researchers from Johns Hopkins University and the University of Buffalo in determining the relationships among pre-pregnancy weight, weight gain during pregnancy, and birthweight. The strong suggestion developing from this research is that current obstetrical practice in limiting a woman's weight gain in pregnancy to control potential toxemia may require reevaluation. Two papers resulting from this investigation have appeared in obstetrics journals, and 5 more are in preparation. These latter discuss such topics as the relationship of maternal weight gain to toxemia and to cigarette smoking, and the effects of changes in weight gain in successive pregnancies of the same woman.

12. Participated in the design of a study with researchers from New York Medical College to determine the extent to which prenatal care affects neonatal mortality.

13. Participated with investigators from the Boston Lying-In Hospital in the design of a study to measure the effects of parity, with the factor "age of gravida," which is usually confounded with parity, held constant.

14. Participated in a study relating head circumference at one year to subsequent evaluation (4-year) of the IQ of a child. Paper giving findings has been submitted to Pediatrics.

15. Participated with consultants specializing in several disciplines in developing a plan for a comprehensive report to the medical community of data arising from the Collaborative Project. The so-called Basic Document should be the first of a series of volumes detailing studies in depth of various subject matters, prepared from the data unique to the Project. The Basic Document is designed to give the frequency distributions and gross associations that are preliminary to these volumes. Data for this first volume currently are being processed.

16. Participated with a staff member of Children's Hospital, D. C., in the design and analysis of a study relating early neurologic signs to later neurologic deficits. Results of this study were reported at a pediatric society meeting in Mexico City, and are currently in preparation for publication.

17. Participated with investigators from New York Medical College in the design of a study to measure recidivism in pregnancy, utilizing data developed from the findings relating to women who were observed in two consecutive pregnancies in the Collaborative Project.

18. Provided consultation and statistical assistance in a study of Herpes Simplex cephalitis in mice. Analyses of contingency tables of mortality, Fisher's exact tests of two binomial groups, and Kolmogorov-Smirnov two sample tests were used to analyze data developed from a study of the effect of intracerebral injection of Herpes virus in mice, following subcutaneous infection with a large dose of the same virus.

19. Consultation was given on a problem arising from the statistical analysis of matched sample data, in a study relating the neuropsychological outcomes of children to the proteinuria of their mothers during pregnancy. An analysis of the data used in this study, employing cautious reasoning, reversed the significance of the result, and led to a conclusion different from that first obtained by the investigator.

20. Participated in the design of a trial, to be undertaken with monkeys, to test relationships between restricted protein and/or caloric intake and weight gain during pregnancy, birthweight, and neurologic and behavioral sequellae in offspring. This study may be conducted as a joint effort of NINDS and a primate center.

21. Provided a discriminant analysis on data for a study of early and late fetal deaths.

22. Participated with researchers from Columbia University and New York Medical College in design of a study to determine how the sociocultural status of an ethnic group (Puerto Ricans) living in a reasonably circumscribed area (upper Manhattan), affects the outcomes of pregnancy.

23. Participated with investigators from New York Medical College in the design of a study to evaluate the effects of retinal hemorrhages in the newborn.

24. Consulted on, and provided specifications for, a study of the significance of chorangiomas in relation to maternal, obstetrical, and fetal characteristics.

25. Consulted on study relating socio-economic factors to survival of infant.

26. Participated in design of a study on relationships among congenital malformations. This study will be reported in a meeting to be held this summer in The Hague.

27. Participated in a study of certain parameters of fetal heart-rate measurements, as they predict neonatal distress. This study will be reported as a chapter in a book on the significance of fetal heart-rate findings.

28. Participated in a study designed to determine the associations of low hematocrit in the newborn to neurological signs.

29. Performed a multiple regression analysis relating birthweight to a wide variety of factors. Findings from this study will be reported at the Pan-American Conference by a member of the Office of Biometry in June, 1969.

30. Designed a study measuring the placental weight/birthweight relationship. Data from this study currently are being subjected to statistical analysis.

31. Participated, with a Columbia University researcher, in the design and analysis of a study relating mental retardation to post-natal events. Results of this study were reported in a meeting of a pediatric society, and will shortly be published in the proceedings of the society.

32. Performed a variance analysis of examiner differences in the 8-year examination, to determine the extent and significance of these differences.

33. Provided consultation, study design, statistical analysis and/or specifications for the following studies:

    a. Associations between serum amino acid levels of gravida and selected fetal outcomes.

    b. Relationship of hematocrit and cord-clamping time to psychometric performance.

    c. Intra-partum deaths.

    d. Relationship of 1- and 5-minute Apgar scores to performance at 4 years.

    e. Comparative mortality among infants, by neurological abnormality.

    f. Premature spastic diplegia.

    g. Neuropsychological outcome of full-term occiput posterior deliveries.

    h. Physical growth of children and gravidae.

    i. Effect of prepartum hemorrhage and treatment with steroids on neuropsychological outcome of offspring.

    j. The effect on the baby of hypertension and proteinuria in the mother.

k. Mortality and morbidity among low weight infants.

l. Frequencey distribution of complaints of gravidae on earliest obstetrical review.

m. Albuminuria in the gravida, and its effect on the child.

INTRAMURAL PROGRAMS

Surgical Neurology Branch

1. Provided consultation and statistical assistance for experiments in the Experimental Head Injuries Studies. A modified bioassay-probit analysis procedure was employed to determine the 10%, 50%, and 90% levels of concussion of concussed and non-concussed monkeys, with direct impact and whiplash-concussion being measured by angular velocity (rad./sec.) and angular acceleration (rad./sec.^{2}).

2. Refereed, and provided statistical analysis for, a paper "The Physical Factors Related to Experimental Concussion," by V.R. Hodgson. All statistics were recomputed and reanalyzed correctly, entailing ANOVA's and t-tests, and an interpretation of results given.

3. Provided consultation, the preparation of the model, the preparation of the computer program (CEIR remote) and statistical assistance on a head injury project relating to blood and cerebrospinal fluid. The analysis included the application of curvilinear-polynomial regression, time series, multiple time series, etc., techniques leading to further analyses by use of orthogonal polynomial, indices of determinations, and regression analyses.

4. Provided consultation, preparation of the mathematical model, preparation of computer programs (Dial Data and Call 360), and statistical assistance on a monkey study relating to Arterial, Venous, and C.S.F. PO_2, PCO_2, and Ph. The analyses included ANOVA's, covariance analyses, t-tests, curvilinear regression techniques (including the testing of truncation or censoring methods) time series analyses, and regression analyses.

5. Provided consultation, preparation of the model, and statistical assistance in the preparation of tables from the data of the Brain Lesion Study in relation to "with Thalamic degeneration with Cortical, Subcortical, and Thalamic destruction and degeneration" and "without Thalamic degeneration with Cortical, Subcortical, and Thalamic destruction and degeneration."

6. Statistical assistance was given in the analysis of electrical response data obtained from contralateral as well as ipsilateral stimulation in the brain. An exact test for 2 x 3 contingency tables was employed. Statistical significance of the result was discussed and interpreted.

7. Reviewed and discussed with Dr. Li two research papers on mathematical and computer analysis of spike-train data. These are "Neuronal Spike trains and Stochastic point processes." I. The Single Spike train and Neuronal Spike trains and Stochastic point process. II. Simultaneous Spike trains. Both are by Perkel, Gerstein and Moore (1967).

8. Consultation has been given in analog-to-digital conversion of Dr. Li's micro-electrode data recorded from the brains of cats. The procedure suggested, although partially manual in nature, does provide useful digital data free from background noise. (The automatic conversion procedure is not available at the present time.)

9. Provided statistical assistance on analysis of data and preparation of computer programs for evaluating inter-spike interval histograms, serial-correlograms, auto-correlograms of single spike-train data and cross-correlograms of two spike-trains recorded simultaneously.

10. Reviewed four papers by Dr. Lansdell on the effect of temporal lobe removals on MMPI and DAT scores for recommendations on future investigations. These papers are "The Meaning of the Taulbee-Sisson Configurational Score on the MMPI with Neurosurgical Patients," "Effect of Extent of Temporal Lobe Surgery and Neuropathology on the MMPI," "Evidence for a Symmetrical Hemispheric Contribution to an Intellectual Function," "Effect of Extent of Temporal Lobe Ablations of Two Lateralized Deficits."

Medical Neurology Branch

1. Consultation was given in a problem in muscle mechanics in the use of regression equations in reverse. If a regression equation $y = a + bx$ is available, the reversed equation for x in term of y can be easily obtained. However, if interval inference of x for some given y is required, the confidence interval procedure can then be quite complicated. Brownlee's procedure was referred for possible use.

2. Reviewed paper on force-velocity on muscle studies, "Series Elastic Element in Rat Limb Muscle," by Jay B. Wells, including review of the statistics used, the write-up of the results obtained, and their interpretation.

3. Provided consultation, liaison with DCRT-LAS and CDP, and statistical assistance in:

    1. Reading films on muscle mechanic experiments

    2. Putting these data on cards or tape

    3. Applying Hill's force-velocity curve transformation to the data

    4. Setting up a mathematical model for analyzing this transformed data

5. Interpretation of results

Experimental Neurology Branch

1. Consultation and assistance were given in the statistical analysis of experimental data and interpretation of results for a correlative histochemical and quantitative study on cerebral glycogen following brain injury in the rat. This resulted in the use of a split-plot type ANOVA and subanalysis to investigate the significant relations between glycogen content in the brain and such factors as time after brain injury and side and location of the brain where injury was induced. An experiment model (partially nested) was developed in formulating statistical analysis procedure. Assistance was also given in reviewing the final manuscript of the research report to be published in the Journal of Experimental Neurology.

Ophthalmology Branch

1. Provided consultation, preparation of computer program (Dial Data), instruction, and statistical assistance to determine the proper curves to fit sets of data on intraocular pressure and changes in the volume of fluid in the anterior chamber of the eye; these included partial and multiple correlations, exponential and curvilinear regression, regression analyses, and co-variance analyses.

2. Gave consultation and statistical assistance on first, the analyses of data on intraocular pressure and aqueous outflow in time intervals before and after drugs including linear correlation and regression analysis and, second, testing the differences between the least squares, and last hour average outflow, average outflow and last hour Inulin, and Probe (average) K-outflow and Risa K-outflow including ANOVA's and paired t-tests.

3. Provided consultation and statistical assistance in checking computations of means and standard deviations on conductive velocity data including phasics, tonics, and late grades.

Neurophysiology Branch

1. Reviewed statistical methodology described in two papers: "The end-plate potential in mammalian muscle," by Boyd and Martin (1956), and "Simple stochastic models for the release of quanta of transmitter from a nerve terminal," by D. Vere-Jones (1966).

2. Consulted on and gave assistance in statistical procedure for analyzing recorded end-plate potential data in comparing two groups of test subjects. This involves elimination by statistical adjustment of drift effect existing during recording.

3. Undertook research to develop a probability model for estimating quanta of transmitter released from a nerve terminal as well as the size of unit quanta from end-plate potential recordings.

4. Data from three experimental animals were used to demonstrate that a particular hypothesis (mathematical model) incorrectly predicts the preservation of the relation between diameter and resistance in severed and normal nerve fibers. A log transformation was applied to the model in order to subject it to statistical testing. The data were transformed analogously and a test was constructed based on the Central Limit Theorem and the combination of independent continuous test probabilities.

5. Consulted on a study of the effect of electrical stimulation on a function of end plate potential. The independent variable in this study is the length of time between successive stimulations. The data consist of quantal readings (a function of end plate potential) corresponding to four separate time intervals. Regression curves have been fitted to the data and we are currently investigating procedures which enable one to compare these curves even if their functional form is different. We are also investigating other statistical methods which provide criteria for the comparison of two time series.

6. Consulted on and gave statistical assistance in the analyses of experimental data in a neuromuscular study involving a problem of applying analysis of variance to small data where error appeared to be heterogeneous.

Electroencephalography Branch

1. Consultation and statistical assistance were given in a clinical-electrographic study of co-existence of focal and bilateral diffuse paroxysmal discharges of epileptics. This involved the comparisons of control vs. experimental groups in the distribution of age of onset of epileptic seizure and the age distribution of the first EEG examination, testing association of focal and bilateral discharges vs. other EEG variables, and statistical effort to eliminate the effect of age from these tests. A review was made of the manuscript submitted for publication.

Laboratory of Molecular Biology

1. The numerical toxonomy procedure of grouping biological objects, developed by Sokal and Sneath (1963) was used to classify bacteria mutants based on their growth response to five cultural media. A Fortran computer program was prepared. Result obtained from this work was sent to Dr. Freese for reference.

Perinatal Physiology Branch

1. Consultation and assistance has been given in data processing, brain lesion recording, and coding procedure in projects dealing with acute asphyxia, hypoxia, and chronic asphyxia studies on rhesus monkeys. Further assistance will be given in analysis of collected data from these experiments.

2. Under control and test settings intragroup or intergroup agonistic behavior was observed in two groups of rhesus monkeys. Of interest are relations between the agonistic behavior frequencies in different group and situational combinations. Statistical procedures involve analysis of variance when the proper conditions are satisfied. Significance tests have demonstrated that analysis of variance conditions are not satisfied in some cases, consequently the appropriateness of certain nonparametric procedures is presently being investigated for the exceptional cases.

3. An experimenter of the Perinatal Physiology Laboratory obtained the organ weights of baby sheep which were sacrificed at birth. Their mothers had been allocated to one of three treatment groups--normal controls; those in whom a section of placental tissue was removed at 50 days gestation; those similarly treated at 90 days. A discriminant analysis of the organ weights of the babies provided perfect prediction of the allocation of the mothers into control or operated groups.

OTHER ORGANIZATIONS

Office of Secretary, DHEW

1. Mathematical assistance was provided for a problem in dyslexia and related reading disorders. This involved the evaluation of the proportion of surplus in the lower side of an observed standard score distribution over the expected (Gaussian) distribution. The density function and the observed distribution were constructed through probit transformation of percentile data and a polynomial regression procedure, permitting the evaluation of the surplus. A computer program was prepared for the computation of the probability function and the density function of the derived distribution.

NICHD

1. The effects of several virus strains on a group of 10 individuals, whose only exposure to influenza had been during the period of the 1918 influenza pandemic, were compared. The experiment consisted of exposing blood samples from the individuals to different virus strains, with the logarithms (base 2) of the resulting antibody levels constituting the data to be analyzed. Since the nature of true distributions underlying the data could not be ascertained, several statistical tests were used to compare the means of the various data sets; these included the Wilcoxon rank sum test, the Fisher randomization test and its approximation, and three variations of the t-test. All yielded essentially the same results.

2. Provided consultation, preparation of a computer program (Dial Data), and statistical assistance for a study of the cerebrospinal fluid of Kuru infected monkeys for an NIH Lab Series and a Contract Lab Series. The analysis included comparisons of the two series, comparisons of

Kuru vs. controls, and comparisons of the components of the cerebrospinal fluid by ANOVA's, covariance analyses, and t-tests.

NCI

1. Provided consultation to the Committee on Contracts, National Cancer Institute, in data processing and computer techniques.

NIGMS

1. Participated as a member of an ad hoc review committee in the site visit of a comprehensive drug utilization program by the Tufts University School of Medicine.

FUTURE PLANS

Some of the material below pertains to program activities. Since the Office of Biometry is a service organization, it must be decided by the Institute whether OB should extend its efforts into program fields.

1. It is obvious, from OB consultation on certain studies, that there are weaknesses and shortcomings in the statistical and mathematical methodologies required to deal with given problems. There is, for example, a need for the derivation of a distribution compounding the Poisson with the Gamma or the Gaussian, and the development of an optimal procedure for estimating the parameters involved in the distribution function; given these, it would be possible to improve the mathematical model describing the probability structure of end plate potential observations in neuromuscular experiments.

Similarly, during the recent OB effort to provide information to the Statistical Perinatal Ad Hoc Committee, a particular analysis was required for which current methods did not satisfy well the theoretical assumptions. OB developed a method which may be more suitable than are current methods; the new method needs to be tested and analyzed, with results published for critical review.

Again, it would be useful to develop a distribution-free technique by which one could discriminate between two populations, without the requirements that the usual assumptions of independence and continuity be satisfied. It may be possible to develop such a technique based on (the possible refinement of) a well known inequality in probability theory.

It would be useful, also, to construct a statistical test to determine whether various sequences of observations were taken from the same or different time series. It may be possible to do this from results that have been developed in the general theory of Markov processes.

The Office of Biometry, then, proposes to devote an effort to the development of statistical and mathematical methodology, mainly for application to the problems it encounters in the field of neurology.

2. The Office of Biometry believes that it would be worthwhile for NINDS to develop a substantial body of epidemiological information on specific neurological disorders, as, for example, stroke. It is prepared to offer full support to any arm of the Institute--say, the Epidemiology Branch--that might undertake a survey to develop such information.

3. A comprehensive drug utilization program is currently being carried out by a group of investigators at the Tufts University School of Medicine, with support from NIGMS.

OB believes this to be an extremely strong program, and feels that it might readily be adapted to the study of the responses of patients with neurological disorders to drug therapy, and of toxic reactions developing from such therapy.

If the Institute determines whether it has an interest in developing data through this program--either in a general survey or for particular drugs, say L-DOPA--OB is prepared to offer full statistical and data processing service and support.

PROBLEMS

In normal circumstances, the Office of Biometry would request additional personnel, for whom there would be a substantial workload. Under current hiring conditions, such a request would be impractical. Moreover, the Office of Biometry has received strong support and assistance from the Institute, to the extent that some of the effects of the current personnel shortage have been alleviated.

PUBLICATIONS

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2. McCracken, G.H., Hardy, J.B., Chen, T.C., Hoffman, L.S., Gilkeson, M.R., and Sever, J.L.: Serum immunoglobulin levels in newborn infants. II. Survey of cord and follow-up sera from 123 infants with congenital rubella. Journal of Pediatrics 74: pp. 383-392, 1969.

3. Jackson, E.: Missing values in linear multiple discriminant analysis. Biometrics 24: pp. 835-844, No. 4, Dec. 1968.

4. Benson, R.C., Shubeck, F., Deutschberger, J., Weiss, W., and Berendes, H.: Fetal heart rate as a predictor of fetal distress. OB & Gyn. 32: pp. 259-266, No. 2, Aug. 1968.

5. Niswander, K.R., Singer, J., Westphal, M., and Weiss, W.: Weight gain during pregnancy and prepregnancy weight. OB & Gyn. 33: pp. 482-491, No. 4, April 1969.

6. Nelson, K. and Deutschberger, J.: Head circumference as a predictor of 4-year I.Q. Pediatrics (in press).

7. Gordon, M., Niswander, K.R., Berendes, H., and Kantor, A.: Fetal morbidity following potentially anoxigenic obstetrical conditions. VII. Bronchial asthma. Amer. J. Obstet. Gynec. (in press).

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Man Years	
Professional	27.8
Clerical	13.6
	41.4

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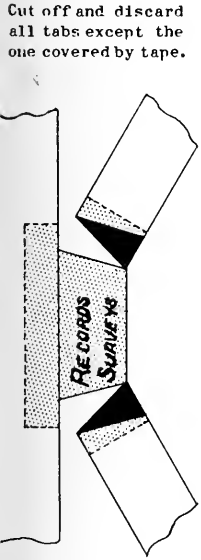

TABBED SEPARATOR SHEET
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(3-56)

SURGICAL NEUROLOGY BR.

BRANCH

DIRECT TRAINING

& NEUROANAT. SC.

TABLE OF ORGAN. IR

SCIENTIFIC DIR.'S RPT. IR

DIR.'S RPT.

MEDICAL NEUROLOGY BRANCH

ANNUAL REPORT

July 1, 1968 through June 30, 1969

Direct Training

National Institute of Neurological Diseases and Stroke
National Institutes of Health

Funds under this activity provide for the support of the Institute's training programs for professional, technical, administrative, and clerical personnel, including job-related training at both government and non-government facilities.

A Commissioned Officer from the Collaborative and Field Research program area is receiving basic training in neurology and epidemiology at the Grady Hospital in Atlanta.

Training in neurology was supported also for Commissioned Officers of the Division of Hospitals at the University of Washington, Seattle and in otolaryngology at Washington University, St. Louis.

Short-term courses are also being supported as funds are available.

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Table of Organization

Intramural Research

National Institute of Neurological Diseases and Stroke
(Personnel on hand May 1969)

Office of Associate Director

Director of Intramural Research - Henry G. Wagner, M.D.
Clinical Director - Maitland Baldwin, M.D., M.S., F.R.C.S.
Administrative Officer - Glenn E. Hammond
Mathematician - Rosalind B. Marimont
Administrative Officer - Elizabeth W. Snowden
Administrative Officer - J. Loring Jenkins
Photographer (Scient. & Tech.) - Wesley Pearson, Jr.
SR Surgeon - John N. Hubbard, Jr., M.D.
Surgeon - Alan L. Norton, M.D.
Special Assistant - Nancy F. Nusbaum
Travel Assistant - Ida M. Chernikoff
Photographer (Medical) - Jerry M. Ellis
Procurement Clerk - Doris R. Perry
Secretary (Stenography) - Margret Shipley
Biological Laboratory Technician - George R. Duvall
Budget Clerk (Typing) - May I. Ferrari
Biological Laboratory Technician - Adrian P. Loftis
Clerk-Stenographer - Lynne H. Minnick
Laboratory Worker - Kenneth M. Oglesbee
Laboratory Worker Leader - Clifford A. Seay
Laboratory Worker - Harold E. Smith

Section on Technical Development

Computer Programmer - William H. Sheriff, Jr.
SA SAN E - Howard M. Seidmand
A SAN E - Raymond M. Fish

Laboratory of Experimental Neurology

Acting Chief - William F. Caveness, M.D.
Secretary (Stenographer) - Dee Ann Lodge

Medical Neurology Branch

Office of the Chief

Chief, Medical Neurology Branch - W. King Engel, M.D.
Associate Neurologist - Dale E. McFarlin, M.D.
SR Surgeon - John R. Warmolts, M.D.
Surgeon - Pieter R. A. Kark, M.D.
Surgeon - Gregory O. Walsh, M.D.
Surgeon - John W. Walsh, M.D.
Surgeon - Darell D. Bigner, M.D.
Surgeon - Paul W. Hathaway, M.D.
Surgeon - Lowell H. Baker, M.D.
Surgeon - William H. Olson, Jr., M.D.
Surgeon - Darryl C. DeVivo, M.D.
Medical Officer - David E. Pleasure, M.D.
Biological Laboratory Technician - Guy G. Cunningham
Chemist - Mary E. Collins
Biological Laboratory Technician - Donald Bishop
Clerk-Dictating Machine Trans. - Patricia D. Williams
Clerk-Dictating Machine Trans. - Lucy L. Riley
Clerk-Dictating Machine Trans. - Gertrude E. Wright
Biological Laboratory Technician - Pauline R. Middleton
Laboratory Worker - Matthew P. Meadows

Section on Biophysical Applications

Surgeon - Nicholas A. Vick, M.D.
Secretary (Stenography) - Vernita Bergmeyer
Physiologist - E. Carolyn Derrer

Section on Neuroradiology

Research Medical Officer - Giovanni Di Chiro, M.D.
Clerk-Stenographer - Janice A. Dawn

Section on Clinical Applied Pharmacology

Super. Research Pharmacologist - Richard L. Irwin, Ph.D.
Research Physiologist - Jay B. Wells, Ph.D.
Biologist - Katherine L. Oliver
Secretary (Dict. Mach. Trans.) - Emma P. Dick

Surgical Neurology Branch

Office of the Chief

Chief, Surgical Neurology Branch - Maitland Baldwin, M.D.
Associate Neurosurgeon - John M. Van Buren, M.D., M.Sc.
Medical Officer - Choh-luh Li, M.D., M.S.
Medical Officer - Ayub K. Ommaya, F.R.C.S.
Medical Officer - Thomas H. Milhorat, M.D.
Research Psychologist (Clinical) - Paul Fedio, Jr., Ph.D.
SR Surgeon - Ronald A. Naumann, M.D.
Surgeon - John F. Davison, M.D.
Surgeon - Robert A. Ratcheson, M.D.
Surgeon - Nicholas E. Grivas, M.D.
Surgeon - Fremont P. Wirth, Jr., M.D.
Surgeon - Howard H. Kaufman, M.D.
Surgeon - Robert L. Grubb, Jr., M.D.
Biologist - Rosemary C. Borke
Biologist - Levon O. Parker
Biologist - Kenneth E. Parker
Supervisory Operating Room Nurse - Frances D. S. Lamberti, R.N.
Supervisory Operating Room Nurse - Mildred L. Haddox, R.N.
Secretary (Stenography) - Maxine O. Reynolds
Operating Room Nurse - Delta H. Trickett, R.N.
Operating Room Nurse - E. Dolores Blessing, R.N.
Operating Room Nurse - Barbara L. Edmonds, R.N.
Clerk (Dict. Mach. Trans.) - Helen M. Andregg
Clerk Stenographer - Linda M. Ingram
Clerk (Typing) - Celeste L. Sewell
Health Technician - Morris M. Williams
Health Technician - M. Arthur Banks
Secretary (Stenography) - Michele Y. Shevitz
Clerk-Stenographer - Dawn M. Broadhurst
Health Technician - Howard F. Stewart, Jr.
Clerk-Stenographer - Cheryl R. Parks
Health Technician - Octavie W. Jacobs
Histopathology Technician - Vernon G. Mastin, Jr.

Section on Primate Neurology

Biologist - Kenneth Rich
Biological Laboratory Technician - Norman E. Mills
Health Technician - Charles E. Sartor
Biological Aid - Leo Jacobs

Section on Neuroanesthesiology

Visiting Scientist - Yushi Kondo, M.D.
Biological Laboratory Technician - Calvin S. Hawkins

Section on Clinical Psychology

Supervisory Research Psychologist - Herbert C. Lansdell, Ph.D.
Secretary (Typing) - Lillian L. Wease

Section on Child Neurology

Medical Officer - Anatole S. Dekaban, M.D.
Surgeon - Michael S. Kappy, M.D.
Research Chemist - Dennis F. Cain, Ph.D.
Research Chemist - George Constantopoulos, Ph.D.
Secretary - Sandra S. Moore
Medical Technician - Jan K. Steusing
Research Chemist - Victoria M. Patton, Ph.D.

Electroencephalography Branch

Office of the Chief

Chief, EEG Branch - Cosimo Ajmone Marsan, M.D.
SR Surgeon - Andrew J. Gabor, M.D., Ph.D.
Surgeon - Russell W. Hardy, Jr., M.D.
EEG Technician (Supervisory) - Rosemarie Schilling
EEG Technician - Alice G. Spencer
Secretary (Dict. Mach. Trans.) - Darlene Yarrow
Clerk (Dict. Mach. Trans.) - Barbara G. Nachman
EEG Technician - Anne M. Moyer

Section on Clinical Neurophysiology

Surgeon - Robert M. Crowell, M.D.
Surgeon - Robert J. Mignone, M.D.
Biologist - John H. Jones

Ophthalmology Branch

Office of the Chief

Chief, Ophthalmology Branch - Ludwin von Sallmann, M.D.
Medical Officer - Vernon G. Wong, M.D.
Surgeon - Richard B. Einaugler, M.D.
Surgeon - Steven M. Podos, M.D.
Surgeon - Thomas F. Minas, M.D.
Surgeon - Richard F. Brubaker, M.D.
Chemist - Patricia A. Grimes
Medical Technician - Eleanor M. Collins
Health Technician - Gerald S. Hoover
Biologist - Richard R. Anderson
Secretary (Stenography) - Rita U. Durant
Clerk (Typing) - Harriett Murphy
Clerk-Stenographer - Anne Karade
Clerk (Typing) - Ethel F. Butts
Biologist - Mercedes M. Saur
Biological Laboratory Technician - Luther R. Dowell, Jr.
Clerk (Typing) - Nancy M. Grams
Clerk-Stenographer - Virginia L. Gray

Section on Ophthalmology Physiology

SR Surgeon - Peter Gouras, M.D.
SA Surgeon - Daniel Finkelstein, M.D.
Physicist - Ralph D. Gunkel, O.D.
Electronic Technician - James L. Jones
Biologist - Mary J. Hoff

Section on Ophthalmology Chemistry

Research Chemist - Marc S. Lewis, Ph.D.
Research Chemist - Ralph J. Helmsen, Ph.D.
Research Chemist - Hitoshi Shichi, Ph.D.

Section on Ophthalmic Metabolism

Research Chemist - Paul J. O'Brien, Ph.D.
Research Chemist - J. J. Bungenberg De Jong, Ph.D.
Chemist - Consuelo G. Muellenberg

Section on Cell Biology

Research Biologist - Arnaldo Lasansky, M.D.
Biological Aid - Jeanette M. Wahler
Biological Laboratory Technician - Julia Matthews

Section on Ophthalmology Pharmacology

Supervisory Pharmacologist - Frank J. Macri, Ph.D.
Biological Laboratory Technician - Joseph G. Brown

Section on Experimental Embryology

Head, Sect. on Exp. Embryology - Alfred J. Coulombre, Ph.D.
Surgeon - Robert L. Trelstad, M.D.
Surgeon - Stephen W. Parker, M.D.
Surgeon - Richard J. Lederman, M.D.
SA Scientist - Joram P. Piatigorsky, Ph.D.
Chemist - Jane L. Coulombre
Medical Technologist - Edna P. McCrane

Laboratory of Neuropathology & Neuroanatomical Sciences

Office of the Chief

Chief, LNNS - Igor Klatzo, M.D., M.Sc.
Surgeon - Andrew A. Zalewski, M.D.
Photographer (Scient. & Tech.) - Edwin W. Moodhe
Secretary (Stenography) - Vivian K. Arnold
Biological Laboratory Technician - Willie Perkins
Biological Laboratory Technician - Albert V. Cantu
Biological Laboratory Technician - Melvin H. Carroll
Clerk (Typing) - Hilda M. Malcolm

Section on Experimental Neurology

MED DIR - Lloyd Guth, M.D.
Physiologist - Herbert Yellin, Ph.D.
Biological Laboratory Technician - Janina Ziemnowicz
Biologist - William C. Brown
Biologist - Phyllis K. Watson

Section on Neurocytology

Research Biologist - Keith C. Richardson, M.Sc.
Research Physiologist - Milton W. Brightman, Ph.D.
Visiting Scientist - Yugve O. Olsson, M.D., Ph.D.
SA Surgeon - Edward Ganz, M.D.
SA Surgeon - William K. Stell, M.D., Ph.D.
Biological Laboratory Technician - Clara E. Mowry

Section on Functional Neuroanatomy

Head, Sect. on Funct. Neuroanatomy - Grant L. Rasmussen, Ph.D.
Medical Officer - Thomas S. Reese, M.D.
Surgeon - Donald M. McDonald, M.D.
Biological Laboratory Technician - Frank D. Nolan
Biologist - Helen J. Osborne

Section on Comparative Neuropathology

Biological Laboratory Technician - Joseph T. Walker, Jr.
Biological Laboratory Technician - Ernestine G. Dye
Visiting Scientist - Konstantin A. Hossmann, M.D.
Medical Technician - Thelma R. Fletcher

Section on Experimental Neuropathology

Medical Officer - Jan Cammermeyer, M.D.
Biologist - Iris Mercado
Biological Laboratory Technician - Margaret G. Johnson
Editorial Assistant - Jane T. Phelps
Biological Aid - Sophia Grabinski

Laboratory of Biophysics

Office of the Chief

Supervisory Res. Physiologist - Robert E. Taylor, Ph.D.
Senior Research Biophysicist - Kenneth S. Cole, Ph.D.
Secretary (Stenography) - Grace C. Ream
Clerk-Stenographer - Constance S. Till

Section on Instrumentation

Electronic Engineer - Leonard Binstock
Electronic Technician - Herbert A. Walters

Section on Mathematical Biophysics

Research Physicist - Richard FitzHugh, Ph.D.
Mathematician - John N. Shaw
Research Physicist - Harold Lecar, Ph.D.
Visiting Fellow - Francisco Bezanilla M., Ph.D.

Section on Membrane Biophysics

Research Physicist (Gen.) - Gerald M. Ehrenstein, Ph.D.

Section on Cellular Biophysics

Research Physiologist - Daniel L. Gilbert, Ph.D.
SR Surgeon - Irving M. Stillman, M.D., Ph.D.
Biologist - Allen D. Spencer, Jr.
Staff Fellow - Harvey M. Fishman, Ph.D.

Laboratory of Neurochemistry

Office of the Chief

MED DIR - Donald B. Tower, M.D., Ph.D.
Surgeon - John E. Franklin, Jr. M.D.
Secretary (Stenography) - Shirley Ann Burdette
Clerk-Stenographer - Carole A. Bromley

Section on Amino Acid Chemistry

SA Surgeon - George S. Allen, M.D.
Chemist - Oscar M. Young

Section on Physiology and Metabolism

Research Chemist - Eberhard G. Trams, Ph.D.
Chemist - Carl J. Lauter
Visiting Fellow - Janice R. Skidmore, Ph.D.

Section on Enzyme Chemistry

Research Chemist - R. Wayne Albers, Ph.D.
SR Surgeon - Frederick J. Samaha, M.D.
SR Surgeon - Allen H. Neims, M.D., Ph.D.
Chemist - George J. Koval
Physical Science Aid - Eunice L. Summers

Section on Lipid Chemistry

Head, Section on Lipid Chemistry - Roscoe O. Brady, Jr., M.D.
Research Chemist - Andrew E. Gal, Ph.D.
Staff Fellow - Richard M. Quarles, Ph.D.
Chemist - Roy M. Bradley
Chemist - Jane M. Quirk
Surgeon - Roger A. Snyder, M.D.
Surgeon - Neal J. Weinreb, M.D.
Biological Aid - Alexander B. Wheaton
Biological Laboratory Technician - Frank J. Fash

Section on Embryological Developmental Chemistry

SR Surgeon - Robert S. Bourke, M.D.

Laboratory of Neurophysiology

Office of the Chief

Act. Chief, Lab. of Neurophysiology - Michelangelo G. F. Fuortes, M.D.
Research Medical Officer - Phillip G. Nelson, M.D., Ph.D.
Medical Officer - Thomas G. Smith, Jr., M.D.
Staff Fellow - George C. Murray, Ph.D.
Surgeon - Gerald D. Fischbach, M.D.
SA Surgeon - Herrman H. Funkenstein, M.D.
Surgeon - Denis A. Baylor, M.D.
Visiting Associate - Peter Winter, Ph.D.
Biological Laboratory Technician - William L. Beane
Secretary (Dict. Mach. Trans.) - June M. Picone
Electronic Technician - Vincent T. Almasy
Clerk-Stenographer - Anita Tana

Laboratory of Neural Control

Office of the Chief

Act. Chief, Lab. of Neural Control - Karl Frank, Ph.D.
Surgeon - David J. Mishelevich, M.D.
Surgeon - Robert E. Burke, M.D.
Scientist - Felix E. Zajac, III, Ph.D.
Research Physiologist - Donald R. Humphrey, Ph.D.
Medical Officer - Charles D. Woody, M.D.
Visiting Scientist - Pedro N. Rudomin, Ph.D.
Secretary (Stenography) - Nell W. Winnie
Clerk-Typist - Joan Paine

Laboratory of Molecular Biology

Office of the Chief

Chief, Lab. of Molecular Biology - Ernst Freese, Ph.D.
Research Chemist - Robert A. Lazzarini, Ph.D.
Research Biologist - Elisabeth G. M. Freese, Ph.D.
Research Medical Officer - L. Robert Berberich, M.D.
Visiting Associate - Kin-ichi Sugae, Ph.D.
Research Chemist - Hans-Jurgen Rhaese, Ph.D.
Surgeon - Charles M. Cashel, M.D.
Surgeon - Robert M. Winslow, M.D.
Chemist - Enid M. Galliers
Secretary (Stenography) - Mary W. Taylor
Mathematics Technician - Naomi K. Boetker
Clerk-Stenographer - Crystal L. Bixler
Biological Laboratory Technician - Blanche E. Lewis
Visiting Fellow - Kiyoi Nakata, Ph.D.
Chemist - Barbara J. Kalbacher

Laboratory of Perinatal Physiology - Puerto Rico:

Chief, Lab. of Perinatal Physiol. - Ronald E. Myers, M.D., Ph.D.
Surgeon - Jerome Engel, Jr., M.D.
SA Surgeon - Stanley B. Holstein, M.D.
Surgeon - Alfred W. Brann, M.D.
SA Surgeon - Chester P. Swett, Jr., M.D.
Surgeon - Harvey M. Shapiro, M.D.
SA Surgeon - Gilbert A. Preston, M.D.
Veterinarian - William D. Thompson, D.V.M., Ph.D.
Surgeon - Robert O. Petersen, M.D.
Medical Officer - Kenneth Kahn, M.D.
Research Chemist - Americo Rivera, Jr., Ph.D.
Research Psychologist - Shum-ichi Yamaguchi, Ph.D.
Research Psychologist - Margaret Varley, Ph.D
Administrative Officer - C. Torres Colondres
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Photographer - Pedro Sola-Amadeo
Chemist - Carmen L. Freixas
Biological Laboratory Technician - Maria L. Alvarado de Yamaguchi
Biologist - Rafael Rivera
Biological Laboratory Technician - Luis C. Gonzalez
Biologist - Luis R. Del Rio
Medical Technician - Maria D. Lopez
Personnel Assistant - Ruby C. Gittens
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Medical Technician - Luis Rivera-Baez
Biological Laboratory Technician - Calixto Jimenez-Lugo
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Biological Laboratory Technician - Esteban Monell-Torrens
Secretary (Stenography) - Paulina T. Papp
Biological Laboratory Technician - Luz Delia G. Salazar
Biological Laboratory Technician - William Rodriguez
Biological Aid - Oscar E. Rodriguez
Secretary (Stenography) - Hilda M. Currier
Biological Laboratory Technician - Gladys Diaz de Torres
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Clerk-Typist - Emelie I. Ortiz
Biological Aid - Rafael Rivera-Correa
Biological Aid - Victor M. Amador
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Clerk-Stenographer - Estebania P. Perez
Messenger & Mail Handler - Alfredo Perez
Guard - Manuel Solla Hernandez
Facilities & Equipment Mechanic - Manuel Maldonado-Rosa
Carpenter - Domisiano Melendez
Carpenter - Heriberto Moreno

Maintenance Worker - Demetrio Melendez
Animal Caretaker - Emilio Tolentino
Maintenance Worker - Aristides Quinones
Maintenance Worker - Pedro A. Robles
Animal Caretaker - Policarpio Quinones
Animal Caretaker - Gregorio Figueroa
Animal Caretaker - Inocencio Robledo
Animal Caretaker - Francisco Acevedo Soto
Laborer - Maximino Reyes Rivera
Animal Caretaker - Bernardino Santana
Laborer - Cayetano Guzman
Laborer - Romualdo Rivera-Rivera
Carpenter - Hipolito Gonzalez
Clerk-Typist - Mercedes Torres-Martinez
Biological Aid - Elba Rosa Colon
Animal Caretaker - Eliezer Melendez-Robledo
Animal Caretaker - Hector L. Vazquez
Laborer - Alejandro Ilarraza

Laboratory of Perinatal Physiology - Puerto Rico - Islands:

SA Veterinarian - Ronald F. DiGiacomo, D.V.M.
Research Biologist - Halsey M. Marsden, Ph.D.
Scientist - Stephen H. Vessey, Ph.D.
Research Psychologist - Elizabeth A. Missakian, Ph.D.
Chemist - Jose Martinez de Jesus
Clerk-Stenographer - Susan J. O'Higgins
Biological Aid - Victor A. Bracero-Pagan
Carpenter - Jacinto Salvador Rosado
Carpenter - Jose A. Flores
Biological Aid - Angel Figueroa-Velez
Animal Caretaker - Israel Cordero
Animal Caretaker - Francisco Ruiz Rivera
Animal Caretaker - Luis I. Hernandez

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CLINICAL DIR.'S RPT.
MEDICAL NEUROLOGY BRANCH

ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR
OF THE
NATIONAL INSTITUTE OF NEUROLOGICAL DISEASES AND STROKE

July 1, 1968 through June 30, 1969

The intramural program has continued to improve and make significant progress. Highlighted here are illustrative facets of this progress. Greater detail will be found in the reports of the Laboratory and Branch Chiefs.

A Laboratory of Experimental Neurology has been added to the intramural program. This activity was formerly a part of the Collaborative and Field Research Program and located in facilities of the Neurological Institute of Columbia University, New York City. When renovation of designated space in the new NINDS-NIMH Building is complete, this effort will be relocated. It is a small effort devoted to study of the relationship of development of the central nervous system and its response to physiological and noxious stimuli. From these studies we expect to formulate data useful in management of clinical conditions that have been disordered by trauma, convulsive states, anoxia, edema, irradiation and other causes.

The new NINDS-NIMH Laboratory Building was completed and accepted by NIH in the early fall of last year. Relocation of the laboratories scheduled to be moved into the new building is well underway. Acceptance of the new facilities by the staff has been excellent. This building offers, in addition to substantial increases in space, many advanced design features for laboratory effort. Thus, in addition to relief from the overcrowded conditions of prior years, the facilities lend themselves to more efficient research.

The Executive Fiscal Control Act of July 1968 curtailed acquisitions so that attrition of personnel occurred. Turnover of total personnel during FY 69 was high and resulted in a net loss in on-board strength from 321 to 311. The turnover has, unfortunately, produced some stress and strain since it was uncorrelated with program priorities or with interdependencies of effort. The number of active projects during the past year was 195 with 32 terminated for various reasons, 29 completed and 71 new projects started.

Collaborative efforts between basic scientists and clinicians of the intramural program, between staff of this Institute and other Institutes and activities were especially rich. A few illustrative examples are the joint effort between the Laboratory of Neurophysiology and the Laboratory of Biochemical Genetics of the National Heart Institute on the properties of nerve cells affected by genetic alterations.

The Neurophysiology staff has for some time collaborated closely with the Office of Mathematical Research (NIAMD) on the properties of the nerve membrane and a very fruitful collaboration existed with the Department of Physics, University of Genoa on developing a model for photoreceptor excitation. The Applied Mathematics Section of DCRT has actively and extensively collaborated with the Laboratory of Neural Control on nerve modeling. This same Laboratory has established collaborative efforts with the Applied Physics Laboratory of Johns Hopkins University and Washington University, St. Louis, Missouri. Our intramural study on head injury is a broad program of interest to the Navy Department and the latter has provided substantial, active joint participation through the National Naval Medical Center, the Naval Ship Research and Development Command and the Naval Air Development Center. Productive work on the hereditary metabolic defect known generally as the Lipidoses is collaborative between the staff of the Laboratory of Neurochemistry and the Molecular Disease Branch of the National Heart Institute.

Our research into the field of cerebral trauma, particularly head injury, has concentrated on testing mathematical models of whiplash injury. The importance of this is that it would provide a basis for determining equivalence between the human situation and animal experimentation. The model under test appears to predict adequately. Additional head injury tests on large primates are in progress. Another aspect of the problem of cerebral trauma is the condition of hydrocephalus. Research on this condition has shown that it is possible to use isotope cisternography as a diagnostic indicator of stasis in the ventricles and thus better define the need for shunting operations. A good experimental model of obstructive hydrocephalus has been produced which manifests itself within about three hours following a procedure for inflation of a balloon placed in the fourth ventricle. Extensive studies are in progress to characterize this model and use it as an experimental tool for further studies of the condition.

Cerebral ischemia is an important focus of effort. In its simplest definition this condition is absence of blood in the brain. The condition commonly arises in shock, cardiovascular collapse, and with massive hemorrhage as in trauma. Absence of blood deprives the brain of its energy supply for metabolic needs and leads to serious and long lasting damage if the condition is not corrected. It has been known that hypothermia will reduce tissue metabolic demands and should provide some degree of protection. A laboratory model has been worked out to study cerebral ischemia in the hypothermic dog. This model permits successful survival 80% of the time following 10 minutes of ischemia and reperfusion. One of the related problems is maintenance and recovery of cardiovascular function and control of blood chemistry. When this is done, the normothermic rhesus monkey will recover completely after more than 20 minutes cessation of blood flow. These studies seem to contradict long standing concepts that brain ischemia for more than five minutes is probably not

compatible with useful survival. Further work is necessary, but clinical implications are obvious. Work has shown that the severe effect of ischemia on the blood brain barrier can be modified by perfusing the cerebral vessels with isotonic oxygenated and glucose free solutions during ischemia. Curiously, the blood brain barrier, normally sensitive to blood flow becomes refractory to barrier damaging agents such as Evans blue if perfused in this way. The blood brain barrier received attention also in an examination of the cyto-architecture responsible. Earlier electron microscopic work (performed here) showed that theendothelium of the brain capillaries is normally not fenestrated as elsewhere in the body. Tight junctions between cells prevent large molecule transfer. The choroid plexus endothelium, however, has been found to be fenestrated but the barrier is formed by the epithelial cells immediately below. The endoneurium on peripheral nerves has been studied and found to contain a similar barrier of junctions of the enclosing endothelial cells.

We are pleased that significant progress has been made in our research on angiographic radiological demonstration of malformations of the arterial blood supply to the spinal cord. It is not only possible to demonstrate diagnostic differences, but the technique will play a major role in operative procedures.

The Ophthalmology Branch has added a section on Experimental Embryology. This section was transferred from the National Institute of Child Health and Human Development. This effort is a welcome addition to the eye research program, since it complements ongoing work and has a rich heritage of past accomplishments in elucidating the development of the cornea, lens and retino-tectal systems. Basic understanding of how the eye sees was furthered by new discoveries on the way retinal receptors and ganglion cells function. Much better detail on the cyto-architecture of the retina is being reported out. Similarly, research on the structure of the visual pigment rhodopsin, the characterization of large proteins in the lens, and the vitreous humor has progressed. Glaucoma, a grave problem of vast complexity, continues to receive attention in basic research. A series of publications has been made on the mechanism of aqueous humor dynamics, which should be helpful in analyzing the action of drugs. Research on the electroretinogram as an aid to the diagnosis of hereditary pigmentary degeneration of the retina has indicated that two types can be differentiated by deficiencies in the cone responses. Another inherited disorder, cystinosis, is receiving attention because it manifests itself frequently in eye pathology. Differences in the two forms of this disorder of amino acid metabolism are being worked out in collaboration with NIAMD. Toxoplasma gondii infection of the eye is a serious condition which was identified and worked out by investigators at NIH. The present studies seek to establish quantitative measures for selecting the preferred drug.

Earlier success with the laminar flow technique as a means of increasing the sterility of the operative field has revealed other application of the technique, such as the patient who requires isolation on the ward. It is one way of removing airborne particles being shed by attending personnel.

Work on the trophic or hormonal influence of nerve continues. Evidence is accumulating that the nerve specifically regulates the metabolism of the end-organ it supplies.

Studies to provide a better understanding of basic neurophysiological mechanisms have been made on the synaptic organization of spinal motoneurons, the activity of auditory cells in the center and optimization of voluntary control of muscle. One focus of concentrated effort has been on noise phenomena in the nerve membrane. These voltage fluctuations reflect molecular events and therefore may well be a key to understanding the excitatory mechanism. Myxicola infundibulum has become routine for voltage clamp studies and should go a long way in replacing the dependence on squid axons which is seasonal and difficult to obtain.

Epilepsy continues to receive a major share of our research effort. One study of the effect of high fat diet in children has shown that it can lead to better control of seizures than has been possible with anticonvulsants. Another effort has been made to research methods for treating patients with intractable epilepsy who are unresponsive to anticonvulsant drugs and not within reach of surgery. This work has shown that the blood brain barrier to drugs like Dilantin is removed during hypothermia and effective and long lasting. Lasting control of seizures in many of these patients can be accomplished. Statistical study of case records is underway to resolve the problem of the patient with seizure disorder but whose EEG does not show typical epileptiform abnormalities. Basic research on animal models of epileptiform convulsive activity produced by agents like strychnine or penicillin examines the mechanism and role of neurones in this type of seizure.

Research has continued on Refsum's disease, Amyotrophic Lateral Sclerosis, Ataxia Telangiectasia, subacute sclerosing encephalitis and brain tumors. In Telangiectasia defective immunoglobulin synthesis has been demonstrated. Hepatic dysfunction is a common factor and a new low molecular weight immunoglobulin has been found. A diet free of phytic acid has been found to lead to a significant improvement in Refsum's disease. Aceto-zolamide is the best prophylactic agent so far found for treating periodic paralysis. Striking improvement has been obtained in myasthenia gravis when treated with ACTH.

The program of studies of the regulatory mechanisms that control RNA synthesis is vigorous and productive of substantial increases in our understanding of these molecular phenomena.

Finally, our program in perinatal brain damage has continued its research on energy deprivation as a cause of cerebral dysgenesis. These studies have shown that fetal asphyxia such as by umbilical cord strangulation produce characteristic changes that vary predictably depending upon duration of obstruction, gestation age, etc. Since severe fetal damage tends to produce still birth, considerable experimentation is required to demonstrate the parameters critical to brain or nerve damage. The present studies have looked at ways of preventing or ameliorating the effects of asphyxia, such as hypothermia, correcting acidosis and the protective effect of barbiturates. Sampling of fetal scalp circulation during delivery has been found to match astonishingly close the values of samples obtained from the central thoracic vessels.

The work on the social structure of free-ranging monkeys is being continued.

It is believed that future progress will continue at a satisfactory pace and significant contributions to the mission will increase.

Serial No. NDS(I)-66-OAD 1460
1. Office of Associate Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Survey of feasibility of visible speech display as an aid for the deaf.

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Man Years:

Total:	.1
Professional:	.1
Other:	0

Project Description:

Objectives: To study the feasibility of using a visual display of speech as an aid in speech training of the deaf.

Methods:

1. Review of status of current visual displays through the literature and consultations.
2. Review of status of research in allied field, such as machine decoding of speech.
3. Analysis of problems encountered to date.

Major Findings:

1. Speech training of the profoundly deaf continues to be difficult and ineffective. Progress in the design of training devices is impeded by a lack of understanding of the speech decoding mechanism. The factors determining both intelligibility and naturalness of speech have not been isolated, and therefore an efficient training scheme cannot be devised.

2. A strong case has been made for early detection and treatment of deafness (Whitnall and Fry, The Deaf Child 1964). It is possible that speech development must take place at close to its normal time of life, or not at all. If this is true, then major attention should be given to mass early detection programs, and training of older children and young adults might be directed toward non-speech language skills, such as reading and writing, speech reading, etc. Further information on this point--i.e. how crucially does age affect the ability to acquire spoken language--is obviously of great importance.

3. There seems to be inadequate communication between the fields of education of the deaf and electronic research which might be applicable,

such as speech decoding, bandwidth reduction, etc. The two fields have substantial areas of commond interest and communication should be encouraged. Bell Telephone Laboratories, which developed the Visible Speech Translator is now reviewing the field and considering further work.

Proposed course of Project

1. To continue the survey of progress in the field, with particular attention to analysis of the problems involved and how NIH can contribute to their solution.

2. To maintain liaison with Bell and other electronics companies whose researches in other areas might contribute to an amelioration of the condition of the congenitally deaf.

Serial No. NDS(I) 68 OAD 1521
1. Office of the Associate Director
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: An investigation of the functional response of the retina to light

Previous Serial Number: Same

Principal Investigator: Henry G. Wagner, M.D.

Other Investigators: Alan Norton, M.D.
Hendrikus Spekreijse, Ph.D.
Myron Wolbarsht, Ph.D.
Harry Blum

Cooperating Units: Naval Medical Research Institute, NNMC, Bethesda, Maryland

Man Years:
Total: 1.7
Professional: 1.5
Other: .2

Project Description:

Objectives: To determine the functional mechanisms in the retina which code the physical parameters of light stimuli to the electrophysiological responses in the neurones of the visual pathways.

Methods Employed: Using eyes of suitable experimental animals, intraretina electrical activity will be detected through conventional biophysical technology and correlated with specified stimuli.

Major Findings: The correlation of intraretinal potentials to moving stimuli has been a promising lead for study. An investigation of the possible role different cells in the retina might play in the mechanism of directional selectivity was undertaken. Extracellular recordings from directionally sensitive ganglion cells were made in order to demonstrate that directional selectivity could be found in the isolated retina. Subsequently, intracellular recordings were made from photoreceptor cells as well as from other cells in the inner nuclear layer of the frog's isolated retina and eye cup preparation of Necturus. No specific directional selectivity, as found in the ganglion cell, could be detected in any of the intracellular recordings. However, the direction of the light stimuli did have

a profound effect of the waveform of one type of response. This response may have originated from either amacrine or bipolar cells.

Proposed Course of Project: These studies will be continued. The use of nonlinear stimuli offers unusual opportunities to explore the information processing in the visual system. This concept will be extended to include changes in spatial position of stimulus as well as chromatic shift to uncover the dynamics of intraretinal actions.

Publications: None

Serial No. NDS(I)-68/OAD 1554
1. Office of Associate Director
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Pure and applied mathematics, with special attention to mathematical models of sensory systems

Previous Serial Number: Same

Principal Investigator: Rosalind B. Marimont

Other Investigators: None

Cooperating Units: Mathematical Research Branch, NIAMD

Man Years:
Total: .5
Professional: .5
Other: 0

Project Description:

Objectives: To conduct research in pure and applied mathematics, with special attention to mathematical models of sensory systems.

Methods: Mathematical Analysis

Major Findings:

1. Models of Visual Function.

Recent study of S potentials in the vertebrate retina by Wagner et. al. at NIH and Svaetichin et. al. elsewhere have tended to confirm the validity of the model proposed in 1962 (Marimont, Rosalind, 'Model for Visual Response to Contrast', JOSA, 52: No. 7, 800-806), in that global and local integration of signals occurs, whereas direct lateral inhibition has not been found. The 1962 model was also found to be related to the Land Retinex model of color vision.

2. Measurement of Action Spectra.

The amplitude of response of an optical system as a function of the wavelength of the incident light is an important characteristic of the system. If some response level is chosen as a criterion, and the input amplitude as a function of wavelength necessary to achieve this level is normalized, the reciprocal of the resulting curve is known as the action spectrum of the system. It is often assumed that the curve so obtained describes a visual pigment contained in the system, and should conform to Dartnall's nomogram.

It is not at all obvious that this curve is an invariant of the system (i.e. does not vary with the response criterion chosen), let alone an invariant of one particular stage of the system. The properties of the system implicitly assumed in the above procedure were investigated. A path of a signal through a visual system may be considered to consist of three parts--an optical path, a transducer, and a nervous system path. It is convenient to refer to these stages as window, transducer, and circuit, respectively. If the input-output response of the circuit can be described for the whole experiment by a single monotonic curve (i.e. no change in the operating characteristics for different wavelengths of input), then one can say that equal outputs from the circuit imply equal outputs from the transducer. If the window is optically linear, and either spectrally nonselective, or of known spectral response, then the input to the window bears a simple relation to the input to the transducer, and the response of the transducer can be derived from that of the whole system. These are sufficient conditions to justify the procedure described. These conditions are not equivalent to univariance (that the neural signal after transduction bears no wavelength information). One must remember that physically, at least in vertebrates, part of the circuit is part of the window, i.e. light passes through various cells of the retina on its way to the receptors. Therefore it is conceivable that the response of these cells could be affected by the wavelength of the incident light, even though their input from the photoreceptors carries no wavelength information. Optical nonlinearities do of course occur in vertebrate systems--the change in pupil size is on obvious one for the whole system, and pigment bleaching is a nonlinearity of the photoreceptors. It is therefore obvious that even in a one pigment system possessing univariance, the measurement described at the beginning of this section may not result in a curve which displays an invariant property of the pigment.

3. General graph and matrix theory.

A previous unpublished paper "System Connectivity and Matrix Properties," was augmented and revised and submitted to the Bulletin of Mathematical Biophysics.

Proposed course

1. To reexamine and refine the visual model proposed earlier, with particular attention to the function of the retina and to try to coordinate this theoretical work with experimental endeavors of my colleagues.

2. To clarify further the theoretical basis of action spectrum measurements, in particular, necessary and sufficient conditions for ascribing the spectrum to a pigment, and ways of determining whether these conditions are met.

3. To continue the study of graphs and matrices and their applications to biological problems.

Publications (in press)

Marimont, R. B.: System connectivity and matrix properties. Bull. Math. Biophysics, 1969.

Marimont, R. B.: Cuttable and cut reducible matrices. J. Res. NBS, 1969.

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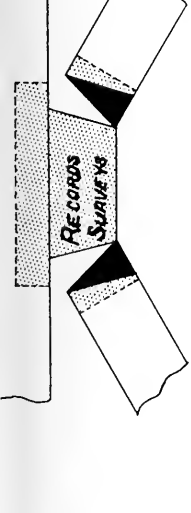

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ANNUAL REPORT
July 1, 1968 through June 30, 1969
Report of the Clinical Director
National Institute of Neurological Diseases and Stroke

Maitland Baldwin, M. D.

The annual harvest from clinical research includes various diagnostic and therapeutic contributions from the several investigators of eye and neurological diseases.

Electroretinographic studies reveal that in circumscribed chorioretinal destruction, response amplitude is reduced, but there is no change in implicit time. However, in hereditary disease, the reduction in amplitude can be accompanied by changes in implicit time.

Specialized angiography of the spinal cord provided diagnostic distinctions between hemangioblastoma and arteriovenous malformations of the spinal cord, thus providing for effective surgical treatment as well. Similarly, isotope cisternography and isotope ventriculography, two diagnostic techniques developed to a high degree in this Institute, have furthered the understanding of communicating hydrocephalus.

Various therapeutic endeavors have yielded gratifying, or even dramatic, results. Chemotherapy for eye infections related to Toxoplasma gondii proved effective, while prednisone treatment in a case of recurrent neuropathy was followed by remarkable recovery. Acetazolamide has proved effective in the prophylaxis of periodic paralysis. In addition, the intradural ligation of vessels feeding spinal arteriovenous malformations has been successfully accomplished thanks to the new diagnostic techniques noted above. Finally, 30 patients with intractable epilepsy have been treated with a new regimen combining intravenous dilantin and cerebral hypothermia. The results are, on the whole, rewarding.

These and other diagnostic and therapeutic contributions were made as part of established projects within which work on fundamental mechanisms of disease has also been done. Our understanding of motor neuron disease, Refsum's disease, myasthenia gravis, and certain myopathies has been advanced. We have more understanding of language mechanisms, and particularly interhemispheral relationships to language in certain disease states.

In addition to investigative responsibilities, all Branches continued under the burden of a heavy service or consultative load. The Eye Service recorded 1,684 requests for consultation fulfilled, and the EEG Service completed 568 examinations from outside the Institute. Medical Neurology replied to 384 consultations.

The outpatient load is also heavy, with a total of 4,483 visits, of which 2,775 were made by Eye patients. This increasing total may reflect the increasing constraints on inpatient census.

536 hours were devoted to operative exposure of the human nervous system (exclusive of preparation and preventive maintenance). The latter required 2,412 hours. 132 hours were devoted to psycho-physiological monitoring of the human nervous system.

The Nursing Units function at a reduced patient census because of nursing personnel ceilings. This restricted census retards the admission rates, increases pressures for admission from the national community, and reduces utilization of the Neurosurgical Suite. Since each Service has now achieved a degree of national recognition, each is under increasing pressure for admission of patients in support of the national medical community. However, the major internal problem (within the Nursing Units) is pediatric allocation. At an average mean total occupancy of 56 (75% of 78 beds), 20 beds are filled by children. Yet these are housed in adult facilities without the specialized logistics that have been developed in modern pediatrics. Both the implementation of special pediatric facilities and the development of modern intensive care units would materially increase patient safety and comfort and the cost-effectiveness of our nursing personnel.

As previously, we are greatly indebted to the Clinical Center for essential support, both from its Directorate and various Departments.

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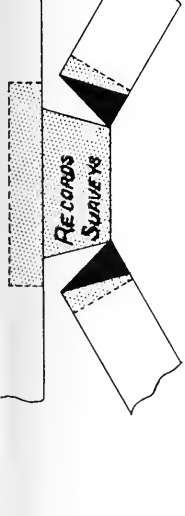

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Annual Report
July 1, 1968 through June 30, 1969
Medical Neurology Branch, IR
National Institute of Neurological Diseases and Stroke

W. King Engel, M.D.
Chief, Medical Neurology Branch

Clinical Investigation Program

Introduction: An inter-related multidimensional attack on the chosen target diseases is emphasized in our application of basic research techniques to clinical neurologic problems. The techniques consist of: histochemistry, biochemistry, immunology, autoradiography, electron-microscopy, tissue culture, and electromyography. In the human neurologic disorders studied, these techniques support thrusts to seek: (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, sub-forms; (c) specific or symptomatic therapeutic response; and (d) animal models of the human pathophysiologic states.

For the clinical investigations, 207 patients were admitted for a total of 5997 patient days, and there were 574 outpatient visits. There were 159 muscle and brain biopsies obtained. The neurologists provided consultations on 384 patients of other departments, performing the indicated myelograms, pneumoencephalograms, and cerebral angiograms.

The one-year approved residency training program in neurology has continued. Fifteen neurologists and 2 technicians came this past year as guest workers to learn clinical research techniques in neurology, especially the application of enzyme histochemistry to human neuromuscular disease. A self-contained audio-visual teaching carrel is being developed, containing programs on general and specific topics of clinical research in neuromuscular disorders.

Two chapters in clinical research texts have been written by invitation on Myasthenia Gravis and Motor Neuron Disorders. Four promising therapeutic programs have been developed.

The Department of Neurology, Warsaw Medical Academy has continued its vigorous collaborative research program in neuromuscular disease under the PL-480 program.

Central Nervous System Degenerations: In ataxia-telangiectasia: the impaired lymphocyte transformation demonstrated was found to be remediable in vitro by a factor from normal serum;

defective IgA synthesis was demonstrated in 12 patients; low molecular weight IgM was present in serum but not saliva; defective production of circulating antibody was demonstrated; hepatic dysfunction was found in 60% of patients, 4 of the affected also having aminoaciduria.

Low molecular weight IgM was demonstrated for the first time in spinal fluid, in a patient with non-neoplastic recurrent encephalopathy; studies indicated it did not result from catabolism of high molecular weight IgM. Low molecular weight IgM also demonstrated as a monotypic variety in a patient with amyloidosis and was further found in about 70% of ataxia-telangiectasia patients and in some myasthenia gravis patients.

In a patient subacute sclerosing panencephalitis (SSPE) and high titers of measles antibody, normal responses were demonstrated to blood group antigen and to skin antigens, and normal lymphocyte transformation, indicating normal cellular defense mechanisms. In another SSPE patient, spontaneous major improvement was documented in the presence of unchanged measles antibody levels (with PRB).

Brain tumors have been reproducibly induced in dogs by intracerebral inoculation of Schmidt-Ruppin Rous sarcoma virus, made possible by an improved method of virus purification and elimination of bacterial contaminants. This reliable and relatively inexpensive animal model is being used (a) to develop the most sensitive animal system in which to attempt recovery of suspected oncogenic viruses from human brain tumors and (b) to improve methods of clinical detection of human brain tumors.

The first and third known families with the neurologic-hematologic syndrome of CNS disease with acanthocytosis and normal β-lipoproteins have been identified (with NHI and NIAMD).

Motor Neuron Diseases: The intolerance to oral glucose in 30% of amyotrophic lateral sclerosis (ALS) patients was found in a similar percent of 4 other groups of neuromuscular disorders and therefore considered not to be disease specific, in contrast to the conclusion of other workers. Studies of tolbutamide tolerance, insulin tolerance, and assay of plasma insulin (with U. Rochester) indicated (a) no consistent abnormality of pancreatic endocrine function in ALS, and (b) loss of muscle bulk is the best explanation of glucose intolerance in the various neuromuscular diseases. Double-blind, controlled clinical trials using quantitative muscle testing were reported as showing lack of therapeutic benefit from: fresh human plasma; vitamin E plus pancreatic extract; lipoic acid; prednisone; and adamantine (an anti-viral agent). Histochemical studies of brain and spinal cord tissue show lack of a specific histochemical change in the motor neurons of ALS and ISMA. In ALS it was

reported that there is no abnormality of the following metabolic processes: synthesis and catabolism of IgA and IgG, catabolism of caeruloplasmin and catabolism of albumin, (with NCI); plasma lipoprotein content, plasma fatty acids, and specifically phytanic acid (with NHI); serum arginine content; aminoacid excretion. Review of our chronic infantile and juvenile (onset before and after age 2 years respectively) spinal muscular atrophy patients demonstrated the former is actually more common, and both appear to be variants of the same (unknown) inherited disorder. Muscle biopsy of very early involved muscle showed that histochemically detectable atrophic fibers were scattered, not in small groups, suggesting fibers of a motor unit are scattered and casting doubt on the physiologic/morphologic concept of a grouped subunit.

Peripheral Neuropathy: In Refsum's disease (hyperphytanic-acidemia) an attempt to circumvent the metabolic defect by a long-term therapeutic diet virtually free of phytanic acid and phytol has been accompanied by statistically significant objective clinical improvement. Electron-microscopy revealed the principal defect in peripheral nerve of Refsum's disease is in Schwann cells, which show (a) hypertrophy and (b) morphologically distinctive intramitochondrial "crystalline" inclusions. A new histochemical method of demonstrating the delicate cytoplasmic processes of Schwann cells in single teased nerve fibers has been reported and applied to events following crush injury of nerve, leading to the hypothesis of a retraction coiling phenomenon of axons.

Prednisone has been demonstrated to be beneficial in a recurrent neuropathy patient not treated until after 11 months of quadriplegia - remarkable recovery occurred, to the point of independent ambulation. With change to an every-other-day steroid program, the patient's improvement has continued (now 17 months after onset of steroid treatment) and side effects reduced. These two aspects of prednisone therapy indicate need for more extensive trials of such a program in this disorder.

Animal models were used to demonstrate: production of selective fiber type hypotrophy with internal nuclei, analogous to a new human disorder identified from this Branch; production of "type grouping" of muscle fibers in animals, a change identified empirically as a sign of chronic denervation in humans, to confirm the pathogenesis proposed from the human data; alteration of muscle fiber histochemical types by suprasegmental lesion (cordotomy) in newborns and adults; a new hypothesis for correlating the physiologic speeding of cross-innervated slow-twitch muscle (soleus) with the induced histochemical fiber types (with LNP).

Axoplasmic flow, demonstrated by autoradiography, is interrupted in cats with neuropathy induced by acrylamide, in contrast to normal and tri-ortho cresyl phosphate induced feline neuropathy, in which proteins move from lumbosacral motor neurons along the ventral roots and from ganglion cells toward the spinal cord along the dorsal roots at about 1.5 mm/day.

Neuromuscular Junction - Myasthenia Gravis: The antinuclear factor in serum from myasthenia gravis patients was found in any or all 3 classes of immunoglobulin, in contrast to the antimuscle factor found only in IgG of both myasthenics and non-myasthenics with thymoma, thereby demonstrating that these two reactivities behave as separate entities. The portion of the IgG molecule responsible for antimuscle factor was shown to reside in the FAB fragment but not the FAC fragment. Treatment of one myasthenic patient with ACTH produced striking improvement, while no change in the absolute lymphocyte count or antimuscle factor titer was documented. Thymocytes from one myasthenia gravis patient underwent transformation *in vitro*, like normal lymphocytes but unlike normal thymocytes.

A facilitating myasthenic response was demonstrated electromyographically in a non-cancer patient with a probable atypical motor neuropathy responsive to long-term steroids and short-term guanidine.

Myopathies, including Dystrophies: Five studies dealt with applying histographic analysis of muscle fiber histochemical types to over 1000 normal and abnormal human muscle biopsies; this approach quantitates changes of diameter of the different fiber types in various diseases.

A program has been developed for a new approach to the overall evaluation of selective versus non-selective changes (size and structural) of muscle fibers in various muscle and nerve diseases.

A new biochemical classification of human myopathies has been proposed to emphasize evaluation of the probability that a given blood or tissue biochemical abnormality plays a significant role in the pathogenesis of the disease in which it is found.

The subtle histochemical abnormalities in muscle biopsies of Duchenne dystrophy carriers have been further delineated.

"Ragged red fibers", characteristic of clinically normal limb muscle in patients with progressive external ophthalmoplegia have been characterized by histochemistry and electron-microscopy on the same specimens.

An animal model source for rods electron-microscopically identical to those of human rod (nemaline) myopathy has been reported.

An apparently new myopathy was identified in identical twins with muscle pain, serum enzyme elevations and lack of ketonuria, all provoked by 24-48 hour fasting. They had excess lipid droplets within muscle fibers histochemically and electron-microscopically. A defect of lipid metabolism is suspected.

A patient with exercise-induced hyperlacticacidemia has been explored in depth and found to have a histochemical/ultrastructural abnormality of mitochondria, though an underlying enzymatic defect remains undetected.

The finding of others that myotonic dystrophy patients have a greatly excessive plasma insulin response without resultant hypoglycemia has been confirmed (with NIAMD).

External trauma has been identified as a source of focal myopathic changes in animals and its possible role in humans discussed.

Idiopathic Inflammatory Myopathy - Polymyositis: An excellent clinical response (one patient becoming clinically normal and remaining so for one year off medication) has been achieved with a therapeutic agent newly applied for this disease - azathiaprine, an immunosuppressive/anti-inflammatory agent. In other patients with this disease, the every-other-day steroid program was shown to be more effective with fewer side effects than the every-day program.

Periodic Paralysis: Acetazolamide (a carbonic anhydrase inhibitor) has been introduced and found to be the best prophylactic agent thus far available in hypokalemic periodic paralysis. It has thus far benefited 9 of 12 patients, two for over 3 years. It has allowed most patients to discontinue potassium therapy and still remain virtually attack-free. Seven of the 12 patients also had moderate or severe persistent proximal weakness between attacks on conventional therapy - with acetazolamide 5 improved, 3 having returned to normal strength. Balance studies and use of other drugs have failed to provide a satisfactory explanation for the mechanism of acetazolamide benefit in this disease.

Hyperaldosteronism was demonstrated in a patient with thyrotoxic periodic paralysis, with reversal of both abnormalities by antithyroid treatment but not with reserpine treatment alone.

Methodology: It was demonstrated that the electron probe can be used to detect otherwise invisible histochemical end-products. Quantitation with the probe indicated that type I

muscle fibers have about 100 times the amount of end-product deposited by the EDTA-activated ATPase reaction as type II fibers. Native (unreacted) sections have only small amounts of intrinsic Ca, P, K, Fe, and Na, and no detectable differences between fiber types.

Individual skeletal muscle fibers were teased from glycerated muscle, histochemically typed, and the same fibers studied for myofibrillar contractile properties. No differences of contractile properties could be discerned between the two fiber types.

Neuroradiology

Radiographic Diagnosis: Selective arteriography has now been performed in 28 cases of arteriovenous malformations of the spinal cord (with SNB). Criteria have been established for the distinction between hemangioblastomas and arteriovenous aneurysms of the spinal cord. The former often present angiographically as discrete areas of "blush" in which no individual vessels are recognizable, a finding not encountered in the latter.

Experimental Spinal Cord Angiography has been carried out in various animals, with the larger experience being accumulated with the rhesus monkey (with SNB; and Diagnostic Radiology, CC). Two new aortographic procedures which allow consistent visualization of the spinal cord vessels in the macaque have been developed: (1) Pressor amine potentiation, and (2) External abdominal compression aortography. Experimental surgical occlusion of major supplying arteries of the thoracolumbar spinal cord in the monkey has been carried out at different anatomical sites: arteria radiculomedullaris magna (ARMM), anterior spinal artery above the ARMM, and anterior spinal artery below the ARMM. Clinical, radiographic and postmortem (including perfusion of microangiographic and neurotropic-fluorescent substances) controls have been performed. A consistent and surprising finding has been that the monkeys remain neurologically intact after the ligation of the ARMM and the anterior spinal artery above the ARMM. On the other hand, the animals become consistently paraplegic if the anterior spinal artery is ligated below the ARMM. These experimental data have augmented, and in some way changed, our understanding of spinal cord arterial supply.

Further experience has been gathered about the syndrome of the so-called "empty sella" and its related clinical conditions (hypo- and dyspituitarism, and spontaneous CSF rhinorrhea). Additional sella turcica measurements of various types of dwarfs have been collected, particularly pertaining to possible effects of growth hormone treatment on the small sella, a condition which is often found in hypopituitaric dwarfs.

Isotopic Diagnosis: The techniques of isotope cisternography and isotope ventriculography have now been used very extensively (with Nuclear Medicine, CC). Particular attention has been concentrated on the evaluation of communicating hydrocephalus of various types, including the so-called "normotensive" or "occult" form. Isotope cisternographic studies carried out before and after establishing surgical CSF shunting, together with clinical follow-up, seem to confirm the original impression that patterns which can be used as prognostic criteria for the treatment of various forms of hydrocephalus do, indeed, exist. In particular, it would appear that early intraventricular penetration of the tracer and persistent and prolonged "pooling" of the radiopharmaceutical in the same ventricles may represent an indication that the patient will benefit, sometimes dramatically, from a CSF shunting procedure.

In collaboration with the Case Institute of Technology, a theoretical analysis of the response of the currently available tomographic scanning systems has been completed. The resolution of the various proposed methods has been determined for variations of the following parameters: collimator focal length, collimator thickness, channel exit and entrance radii, and channel or collimator inclination with respect to the body normal. These data indicated the need for new concepts and have led to the design of a new device, the Tomoscanner. The heart of the Tomoscanner is the collimator system which consists of a series of tapered slits angled to the plane of interest, with a scintillation crystal at the slit exit. Each plane of interest is examined by a set of slits, with each slit tilted in opposite directions from the normal to the plane. The collimator movement is a linear sweep, rotation through an angular increment, and repeat of the motion. Readout consists of film integration of a line sweep on an oscilloscope corresponding to the collimator motion. Construction is simple, and the design incorporates the capacity to examine several layers simultaneously. Experiments to confirm the validity of the concepts on which the design of the Tomoscanner is based are being carried out. If the experimental results match and sustain the original theoretical studies, a prototype Tomoscanner will be built.

Applied Pharmacology

Excitation-contraction Coupling: Ionic mechanisms underlying the contractile function of muscle have been further investigated. Previous results from this work have shown that the movement of Na^+ ions is one of the sequential steps between excitation and contraction. Evidence obtained during the past year shows that relaxation of amphibian slow-type (tonic) skeletal muscle depends on an outward active transport system for Na^+ ions. Experimental conditions which either inhibit the Na^+-K^+ activated ATPase of

muscle or block outward active transport of Na^+ preclude slow muscle from staying relaxed and prevent relaxation after contraction. This finding further emphasizes the importance of the function of Na^+ during the excitation-contraction-relaxation cycle. Temperature coefficient and enzyme inhibition studies indicate that slow muscle relaxation depends on enzymic controlled chemical reactions. Contraction occurs unchanged with inhibited ATPase or blocked active transport of Na^+. Tension develops without temperature dependence, and the rate limiting event in slow muscle made to contract by Ca^{++} depletion is diffusion of ions. A study of the relaxation which follows tension development produced by reducing ionic strength while maintaining osmolarity showed that relaxation was independent of the penetrant properties of either external anions or cations. Tension development was found to be independent of the penetrability of the external anions but required a penetrant cation or reduced external ionic strength. The evidence obtained during the last year has permitted for the first time a rather complete description of the relationship between the physiologically present external ions and the excitation-contraction-relaxation cycle in slow-type skeletal muscle. The dependency of relaxation on enzymatic activity transport is neither a general phenomenon that occurs in all types of muscle tissue nor a unique one that is present only in slow striated muscle, since it does not occur in twitch muscle but does occur to some extent in mammalian smooth muscle.

Mechanical Properties of Mammalian Muscle: Experimentation on adaptative and trophic mechanisms which influence skeletal muscle mechanics were continued along two routes of investigation. Muscles deprived on environmental mobility and hence made atrophic showed a substantial decrease in capacity for tension development with moderate loss of mass (wet weight). Rate of tension rise, intrinsic shortening velocity and the dynamic constants of the force-velocity function were not appreciably changed with 4-5 weeks of isometric immobilization. The elastic element, which is (conceptually) joined in series to the contractile element and together comprise the active muscle, did not change character after immobilization.

In a study of the effect of transferred innervation, the motor axons which supplied fast twitch muscle were made to reinnervate slow twitch muscle in order to effect a change in mechanical response. That muscle significantly increased the amount and rate of contractile energy output. The modified energy output resembled that observed in more rapidly contracting muscles. The experiments indicated that the neural influence is on the active contractile element rather than on the passive components of the contractile apparatus.

In fast and slow twitch muscles of four species of mammals, rate constants of contractile energy output were derived by

applying a widely used mathematical expression which relates the contractile parameters of force and shortening velocity. These constants showed good correlation with the corresponding speed of contraction and may serve to quantify the contractile speed of individual muscles. Moreover, the results indicate that such an index can be derived from isometric contractile responses which are obtained with relative ease. These analyses of contractile speed of different muscles will be correlated with their histochemical fiber type composition.

The elastic energy output of active muscle has been investigated and found greater than that predicted by the current concepts of muscle contractile models.

Serial No. NDS(I)-62 MN/OC 915(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Histochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Michael H. Brooke, M.D.
George Karpati, M.D.

Other Investigators: John A. Morgan-Hughes, M.D.
Norman Robbins, M.D.
Allan C. Stam, M.D.
Jay B. Wells, Ph.D.
Michel Resnik, M.D.

Cooperating Units: Laboratory of Neurophysiology, NINDS
International Post Doctoral Fellowship Program of NIH

Man Years

Total:	2.2
Professional:	2.2
Other:	0.0

Project Description:

Objectives: (1) To study the cellular and subcellular localization of a variety of histochemical reactions in normal human skeletal muscle, neurons and peripheral nerves and to see how they are altered in neurologic diseases. (2) To develop new histochemical techniques and to analyse mechanisms of the reactions. (3) To correlate histochemically defined types of muscle fibers with physiologic, ultrastructural, and biochemical properties. (4) To identify selective or non-selective involvement of one histochemical fiber type in the various neuromuscular diseases. (5) To derive a method for quantitating the findings in a muscle biopsy so that the abnormality may be expressed simply and numerically, in the same way that the results of electromyography may be expressed numerically, and to apply such quantitated

results to give information which may be used diagnostically. (6) To use the electron probe for expanding the boundaries of enzyme histochemistry.

Methods Employed: Histochemical techniques for a number of reactions are being done on muscle and nerve tissue - they have been outlined in previous reports.

Histograms were constructed of the transverse diameter of muscle fibers in a biopsy. At least 200 fibers in each muscle biopsy specimen of more than 1000 patients were analysed. From the histogram the following figures were derived for each fiber type: (1) average diameter and standard deviation, (2) a set of numbers, derived mathematically and representing the proportion of fibers which were abnormally small and those which were abnormally large. The ratio of type I fibers to type II fibers was also calculated.

From human muscle biopsies, after glyceration, individual fibers were teased. From the same single fiber, a portion was typed histochemically, another part studied physiologically, and a third portion fixed for electron-microscopic examination. Correlations of the 3 approaches were made.

Human skeletal muscle tissue was incubated using the histochemical technique developed in this Branch for EDTA-activated ATPase of muscle fibers but only to the point of invisible $CaHPO_4$ precipitate. These sections were then analysed with the electron probe microanalyser (Norelco Model AMR/3, Philips Electronic Instruments) for the amount of Ca and P deposited in the fibers and compared with serial sections of the same tissue incubated in additional cobalt and sulfide solutions to obtain a colored end-product. Electron-beam scanning displays were made for characteristic X-rays of elements in the tissue: sample current and back-scattered electrons were also analysed. Averaged countings over a 1μ spot were taken for the two histochemical fiber types. Native (unreacted) sections were also analysed with the electron probe for intrinsic amounts of Ca, P, Fe, K, and Na.

Various animal models were prepared to study the effects of different neural lesions on the histochemistry of skeletal muscle, as described in the Major Findings. Some of these models were also studied by physiologic techniques to evaluate contractile properties of the intact muscle in vitro for correlation with histochemical results.

Patient Material: Biopsies were obtained from Medical Neurology Branch patients as well as from consultation patients; patient biopsy material was also submitted by outside physicians.

Major Findings: Histochemistry of muscle biopsies continues to provide important information in neuromuscular diseases. The use of histograms as part of the total evaluation of muscle biopsies in various diseases revealed that the two histochemical fiber types behave differently under various conditions, reaction differences not easily apparent without histograms. The following were considered to be the items of "new" information from the histogram analyses: (1) type II fibers are smaller in normal females than in males. They appear to increase in size in association with work and to decrease in size following inactivity. This inactivity may be due to functional or organic causes ranging from bed-rest to corticospinal tract disease. The type I fibers are not thus affected. (2) The characteristic finding of chronic familial peripheral neuropathy in adults and denervating diseases in children is the presence of large type I fibers. This change differentiates these conditions from simple peripheral neuropathies in adults and from ALS. (3) Approximately 80% of patients with myotonic dystrophy have preferential atrophy of type I fibers in the biceps and vastus lateralis. None has these changes in the gastrocnemius. (4) Myasthenia gravis shows a reduction in the size of both fiber types but the type II fibers were more affected than the type I's. (5) In periodic paralysis there is no alteration in the size of the type I fibers but there is type II fiber atrophy in about half the biopsies. (6) Duchenne dystrophy is characterized by type I fiber predominance. (7) In floppy babies certain changes in biopsy consisting of small type I fibers are found in those with muscular difficulty not associated with central nervous system damage and hence a better prognosis, but are not seen in those with CNS damage and hence a poorer prognosis. In addition to the above, the classical concepts of muscle pathology were given numerical confirmation (such as the variability of fiber size without twin peaks in the myopathies as contrasted with the twin-peak curves of atrophy and hypertrophy seen in denervation).

A new program for the analysis of muscle fiber reactions in various neuromuscular disorders has been devised. It is based on knowing the histochemical type of the muscle fibers involved and spared in each disease. The program asks whether the muscle fiber pathology reveals changes (a) preferentially affecting one fiber type or (b) indiscriminately affecting both fiber types. Muscle fibers have properties related to their differences, as demonstrated histochemically, which probably represent metabolic differences as well as physiologic differences. However, they are all muscle fibers and thus have certain properties which are the same. It is proposed that there are type I and II lower motor neurons which have different metabolic and physiologic properties. Since they are all motor neurons, they also have certain properties that are the same. Accordingly, various neuromuscular diseases can be conceptualized in regard to (a) whether their muscle fiber

histochemical reactions are determined by properties of the muscle fibers or of the lower motor neurons and (b) whether the muscle fiber reactions are related to the sameness or to the differentness of muscle fibers or of lower motor neurons. If the muscle fiber reaction is related to a sameness or differentness property of the muscle fibers it does not necessarily mean that the basic disease is mainly a disorder of muscle (i.e., a myopathy), but at least theoretically, it could be either a lower motor neuron disease (neuropathy) or a muscle disease.

The effect of suprasegmental lesions on muscle was evaluated following high thoracic cordotomy in adult and newborn guinea pigs. In the adult, moderate atrophy of both histochemical fiber types developed in the gastrocnemius (in contrast to greater atrophy of the type II fibers which resulted from total peripheral nerve denervation). In the adult soleus, normally containing only type I fibers, type II fibers appeared 3-6 months after cordotomy; moreover, in some "myopathic-like" changes occurred. Cordotomy of the newborn prevented the soleus muscle from undergoing the normal maturational change from its neonatal composition of a histochemically mixed muscle (both type I and II fibers) to being uniformly of type I fibers at 6 weeks of age.

Abnormally large groupings of like histochemical fiber types, in contrast to a more evenly mixed mosaic pattern, has been found by us to be an empirical sign of chronic denervation in human disorders and is thereby useful diagnostically. The proposed mechanisms, collateral branching and reinnervation of adjacent muscle fibers from remaining nerve fibers, has received strong support by the production of type grouping in the experimental animal by partial nerve damage and documented sprouting of the intact nerve fibers.

A "paramodel" has been produced by a human neuromuscular disease first described from this Branch, "type I muscle fiber hypotrophy with central nuclei". Rat gastrocnemius muscle denervated at birth showed at 21 days virtually complete hypotrophy of type II muscle fibers with essentially normal type I fibers. The type II fibers were thus more dependent on their nerve supply for maturation. Because the pattern is the same as in the human disease a similar neurogenic pathogenesis is suggested for the latter. The fact that the hypotrophied fibers were type II in the rats and type I in the human disease might be reconciled by species differences in the normal embryonic pattern of maturation of the two fiber types.

The physiological-histochemical interrelationship of skeletal muscle fibers were analysed 5 months after cross-innervation of slow twitch skeletal muscle fibers (soleus) by a nerve (common

peroneal) normally supplying fast twitch muscles. The physiologic data were consistent with the hypothesis that all fibers within the cross-innervated soleus are partially "speeded"; and the data were not compatible with the hypothesis that some fibers in cross-innervated muscle are completely "speeded" to become like normal fast fibers while the other remaining completely slow. The correlations of percent of induced type II fibers by cross-sectional area with twitch time-to-peak or 5/sec tetanus:twitch ratio led to the interpretation that with the ATPase reaction, change in histochemical fiber type occurs when physiologic "speeding" exceeds a certain threshold, viz. "histochemical turnover point".

A technique has been developed for studying the physiologic, histochemical, and electron-microscopic properties in the same individual, teased, human glycerated muscle fiber. It has been demonstrated that histochemical typing of such fibers is possible. When histochemical type I and type II glycerated fibers were studied mechanically, no differences were found to persist in maximal tension, maximum rate or tension development, maximum unloaded shortening velocity, onset of tension development, and compliance-corrected elastic shortening. Only the values for maximum tension reasonably corresponded to in vivo data. If actomyosin-dependent mechanical rate differences exist in type I and II fibers, they could not be domonstrated in glycerated human skeletal muscle fibers.

The histochemical and electron-microscopic findings were correlated in mouse muscle fibers during regeneration following focal cold injury. There was good agreement between the amount of damage and recovery in the various subcellular elements of muscle fibers and the degree of histochemical staining by reactions attributed to those elements (e.g. myofibrils or mitochondria).

It was clearly demonstrated that the electron probe can be used to detect otherwise invisible histochemical end-products. Rough quantitation with the probe indicated that type I muscle fibers have about 100 times the amount of end-product deposited by the EDTA-activated ATPase reaction as type II fibers. Native sections have only small amounts of intrinsic Ca, P, K, Fe, and Na, and no detectable differences between the two fiber types.

Histochemistry of motor neurons and peripheral nerves is described under the ALS Project.

Significance to Bio-Medical Research and the Program of the Institute: It is hoped that the standardization and quantitation of muscle biopsy findings will allow the more accurate interpretation of biopsies from patients with undetermined diseases.

Of particular interest are the findings of an apparently selective involvement of one histochemical type of muscle fiber, suggesting that the particular characteristics of metabolism of that type of muscle fiber are more susceptible to a given disease process. This permits us to ask why they are more susceptible and conversely, what makes the others more resistant? Detailed analysis of single teased human muscle fibers will provide more information on this question.

Animal models for type grouping and for type II fiber hypotrophy with central nuclei provide experimental support for pathogenic mechanisms deduced from human neuromuscular disease. The animal model of the cordotomy effect on muscle and histochemical-electron-microscopic correlation of muscle fiber regeneration provide a basis for evaluating similar changes in human diseases.

Ability to detect elemental precipitates with the electron probe (applicable to all elements with the atomic number of boron, 5, or above) can reduce the number of steps in many histochemical reactions, as in the one described, thereby lessening the danger of false localization. Of perhaps greater importance is that development of new histochemical reactions with otherwise invisible elements in the reaction product is possible using the probe for analysis.

Proposed Course of Project: The detailed results of histochemical and cytochemical evaluation of muscle biopsies from about 1500 patients, averaging 10 different histochemical reactions each, are being compiled into a book Muscle Histochemistry, due to be completed soon. Further correlations of the results from light-microscopy with those of electron-microscopy, single fiber physiology, and biochemistry will be made in human neuromuscle diseases. Other animal models of human neuromuscular disorders will be produced and studied. Additional reactions will be studied with the electron probe.

Honors and Awards: None

Publications:

Brooke, M.H., and Engel, W.K.: The histographic analysis of human muscle biopsies with regard to fiber types. 1. Adult male and female. Neurology 19: 221-231, 1969.

Brooke, M.H., and Engel, W.K.: The histographic analysis of changes in fiber types in disease. 2. Diseases of the upper and lower motor neuron. Neurology 19: 378-393, 1969.

Engel, W.K., and Karpati, G.: Impaired skeletal muscle maturation following neonatal neurectomy. Develop. Biol. 17: 713-723, 1968.

Engel, W.K., Resnick, J.S., and Martin, E.: The electron probe in enzyme histochemistry. J. Histochem. Cytochem. 16: 273-275, 1968.

Karpati, G., and Engel, W.K.: Type grouping in skeletal muscles after experimental reinnervation. Neurology 18: 447-455, 1968.

Karpati, G., and Engel, W.K.: Correlative histochemical study of skeletal muscle after suprasegmental denervation, peripheral nerve section, and skeletal fixation. Neurology 18: 681-692, 1968.

Robbins, N., Karpati, G., and Engel, W.K.: Histochemical and contractile properties in the cross-innervated guinea pig soleus muscle. Arch. Neurol. 20: 318-330, 1969.

Brooke, M.H., and Engel, W.K.: The histographic analysis of human muscle biopsies with regard to fiber types. 3. Myotonias, myasthenia gravis and hypokalemic paralysis. Neurology. In Press.

Brooke, M.H., and Engel, W.K.: The histographic analysis of human muscle biopsies. 5. Children's biopsies. Neurology. In Press.

Serial No. NDS(I)-62 MN/OC 917(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Biochemistry Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Martin A. Hatcher, M.D.
Pieter R.A. Kark, M.D.

Other Investigators: Paul W. Hathaway, M.D.
John P. Blass, M.D.
Daniel Steinberg, M.D.
David M. Fried, M.D.

Cooperating Units: Molecular Disease Branch, NHI
Rehabilitation Department, CC

Man Years

Total:	1.6
Professional:	0.6
Other:	1.0

Project Description:

Objectives: (1) To seek and analyse biochemical abnormalities of neuromuscular and other neurologic diseases; (2) to elaborate biochemical differences and similarities between "red" and "white" muscle of animals and between type I (similar to "red") and type II (similar to "white") individual muscle fibers of humans and animals.

Methods Employed: Blood and muscle biopsy extracts of patients were studied with a variety of techniques:

a. Lactate, pyruvate, creatine phosphokinase (CPK) of blood.

b. Enzymes of glycogen metabolism in muscle - phosphorylase, glycogen, amylo-1,6-glucosidase, phosphoglucomutase, and acid and neutral maltase.

c. Metabolism of muscle studied by incubating various C^{14} labeled substrates - glucolytic and Kreb cycle intermediates, fatty acids, and amino acids - with human muscle homogenates and measuring the amounts of labeled CO_2 produced.

d. Standard isotope techniques for oxidation of succinate, β-OH-butyrate, palmitate, and pyruvate have been adapted to tissue slices of small biopsy specimens of muscle, and techniques for the first three. plus a method for cytochrome oxidase, applied to muscle mitochondria isolated from small specimens.

e. Assays for serum and tissue phytanic acid were as previously recorded (Steinberg et al., Ann. Int. Med., 66: 365-395, 1967).

Patient Material: Selected Medical Neurology Branch patients with neurologic diseases.

Major Findings: a. A new classification of biochemical abnormalities in neuromuscular disorders was presented. This classification has only two dimensions. The first dimension, 'Specificity for Disease', is easier to determine than the second, 'Importance to Cellular Malfunction'. A 'specific' abnormality is one occurring in only one disease. While 'abnormality' is based on comparison with normal controls, 'specificity' must be based on comparison with a reasonable number of disease controls. Since every possible disease control can never be done, terms in this category are potentially subject to being downgraded. An 'unusual' abnormality is one occurring in only a few diseases. A non-specific abnormality is one occurring in many diseases. In each of these three categories, it must be determined further whether the biochemical change is 'important', meaning that it is responsible directl y or indirectly for cellular malfunction, or 'unimportant'. Thus, correction of an abnormality defined as 'important' to the pathophysiology will improve cellular function or restore it to normal, but correction of an 'unimportant' biochemical abnormality will not improve cell function. Correction of a biochemical abnormality could be of therapeutic significance only if it is in the 'important' category, regardless of whether it is non-specific, relatively unusual or specific. This classification of biochemical abnormalities in human neuromuscular diseases may be controversial, in regard to both the catagories used and placement of the various abnormalities in the categories. Whether an abnormality is specific, unusual, or non-specific generally will not be as disputed as whether it is an important or unimportant factor in cellular malfunction. It would be very stimulating if each report of a biochemical abnormality in human neuromuscular disease would contain a 'best guess' regarding the disease specificity and pathophysiologic importance of that abnormality.

b. Further studies were performed on a patient suspected of having a biochemical defect in lactate metabolism associated with abnormal mitochondria by electron-microscopy. A variety of physiological studies were performed involving exercise with concomitant measurement of lactates, pyruvates, glucose, and respiratory quotients. He appears to have exercise-induced hyper-lacticacidemia; whether it is overproduction or under utilization is being determined. An assay was set up to determine the 'normal' reaction of human muscle homogenate when incubated with various C^{14}-labeled substrates - glycolytic and Krebs cycle intermediates, fatty acids, and amino acids. When this test is standardized, it will be applied specifically to the muscle of the above-mentioned patient in order to further define his metabolic abnormality, and it will also be applied to a variety of other biopsies serving as 'disease controls'.

c. In twin sisters with a new metabolic myopathy, clinical biochemical studies and muscle enzyme assays, together with enzyme histochemistry and electron-microscopy, suggested an abnormality of lipid metabolism (see Myopathy Project).

d. Studies have been initiated (with NHI) to analyse slices from small specimens of guinea pig red muscle (pure type I fibers) and white muscle (virtually pure type II fibers), with these radioisotopic assays now appearing to be reproducible: oxidation of succinate, β-OH-butyrate, palmitate, and pyruvate. From these pure red and white muscles, mitochondria have been isolated and the following radioisotopic assays of them now appear to be reproducible: oxidation of succinate, β-OH-butyrate, and palmitate. A method for cytochrome oxidase is also being used.

e. Two patients with Refsum's disease (hyperphytanicacidemia) are on a long-term therapeutic program devised by Dr. Daniel Steinberg (formerly of NHI). It consists of a diet virtually free of the fatty acid phytanic acid and its precursor phytol, because the patients' metabolic defect (possibly a failure of initial alpha-oxidation of phytanic acid) results in large accumulations in blood and tissues of phytanic acid, which may in turn be responsible for the prominent neurologic defects. The special diet has resulted in major reduction of blood phytanic acid and reduction of phytanic acid in fatty tissue (with NHI). There has been a significant but not as striking improvement in neurologic status, documented by careful quantitation (with Rehabilitation Department, CC) of muscle power, motor skills, motor nerve conduction velocity, and other clinical parameters of neurologic function. Although the clinical improvement is temporally related to the therapeutic diet, a more prolonged study period (still being maintained) will be required to clarify that possible relationship.

f. A disposable modified Vim needle is being used to obtain a simultaneous 'closed' biopsy from the muscle contralateral to the one sampled by open biopsy. The two samples are being assayed in parallel to learn whether needle biopsy will provide satisfactory tissue for biochemical studies. If so, it would allow easier sampling and permit several different muscles to be sampled from one patient.

Significance to Bio-Medical Research and the Program of the Institute: Elucidation of biochemical abnormalities, particularly in the realm of enzymes and other proteins, is important to the understanding of neurologic and neuromuscular diseases, and in seeking means of therapy. Putting the various abnormalities in proper perspective regarding pathogenesis and disease specificity, as with the classification proposed, is often neglected but is vital to planning future investigative and therapeutic procedures. Having comparative studies of biochemical differences between type I and type II skeletal muscle fibers will help provide a basis for understanding the pathogenesis of the many neuromuscular disorders preferentially affecting one of these fiber types, as detailed in the Histochemistry Project. Treatment to specifically eliminate from the diet substances improperly handled by patients with concomitant metabolic and neurologic disease, if successful, will place hyperphytanicacidemia (Refsum's disease) along side other metabolic-neurologic disorders, e.g. phenylketonuria, galactosemia, and porphyria, which are treated using the same principle.

Proposed Course of Project: The above techniques will continue to be perfected and applied to muscle from a variety of human neuromuscular disorders to seek further details of biochemical defects therein, as well as the biochemical differences and similarities of human type I and type II fibers. Treatment of hyperphytanicacidemia will be continued.

Honors and Awards: None

Publications:

Engel, W.K., and Hatcher, M.A.: Evaluating significance of biochemical abnormalities in inherited neuromuscular disorders. Proceedings of the 2nd International Congress of Neuro-Genetics and Neuro-Ophthalmology. In Press.

Lazarus, G.S., Fullmer, H.M., Oliver, C.H., Multz, C.V., Barth, W.F., Decker, J.L., and Kamin, E.J.: Collagenase activity of human synovium. Proceedings of the IV Inter-American Congress of Rheumatology. Excerpta Medica Foundation, International Congress Series 143: 19, 1967.

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1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Myopathies

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Darryl C. DeVivo, M.D.
Paul W. Hathaway, M.D.
Nicholas A. Vick, M.D.

Other Investigators: Phillip Gorden, M.D.
Robert C. Griggs, M.D.
Gregory O. Walsh, M.D.
David S. Dahl, M.D.
David E. Pleasure, M.D.
Charles J. Glueck, M.D.
Robert I. Levy, M.D.

Cooperating Units: Diabetes Section, Clinical Endocrinology Branch, NIAMD
Section on Lipoproteins, Molecular Disease Branch, NHI

Man Years

Total:	3.0
Professional:	2.7
Other:	0.3

Project Description:

Objectives: To more fully elaborate the clinical, histochemical, biochemical, and ultrastructural abnormalities of patients with the various myopathies. To further sub-classify patients in each category using those techniques. To treat these disorders by different methods in order to learn which is most effective within each disease category. To produce animal models of pathogenic phenomena.

Methods Employed: Most of the details of techniques applied to human material are described in the various methodological

projects of this Branch. The effects of external pressure on guinea pig muscle was analysed histochemically. Therapeutic trials were conducted with very careful clinical and laboratory examinations to document change of disease state and to identify side-effects at the earliest possible time. In one patient, in addition to the usual clinical, laboratory and pathologic studies, detailed electrolyte balance studies were done to document renal magnesium wasting. Evaluation of several parameters of insulin response were done in collaboration with NIAMD.

Patient Material: Medical Neurology Branch patients.

Major Findings: Identical twin girls were found to have an unusual clinical syndrome of aching muscles and general nausea provoked by 24-48 hour fast, with marked rise of "muscle enzymes" in the blood and occasional myoglobinuria following such episodes. Presence of excessive lipid droplets found by histochemistry and electron-microscopy in otherwise normal muscle fibers suggested a defect in lipid metabolism, as did the observation that the patients did not produce the normal ketonuria expected after the 48 hour fast. (Studies of their carbohydrate metabolism were normal.) A specific enzyme defect has not been demonstrated, but the findings suggest this is a new myopathy based on a lipid metabolism defect, analogous to the several known defects of carbohydrate metabolism (e.g. phosphorylase deficiency) that give rise to specific myopathies.

In idiopathic myositis (polymyositis, dermatomyositis) a therapeutic regimen of every-other-day prednisone treatment has been evaluated on a modest number of patients and shown to be more beneficial and have fewer side effects than the every-day program. The introduction of azathiaprine, an immunosuppressant/ anti-inflammatory agent, for the very effective treatment of dermatomyositis provides alternative medication to steroids and demonstrates a new approach to the treatment of this group of myositis, as detailed in the Immunology Project of this Branch.

In myotonic dystrophy, other investigators have reported very high plasma insulin responses to orally administered glucose. With NIAMD, this response has been confirmed. In 7 of 12 myotonic dystrophy patients, the insulin response markedly exceeded the normal range. A markedly exaggerated insulin response was also observed after arginine stimulation but not after tolbutamide. The patients exhibited nearly normal sensitivity to exogenous and endogenous insulin, and no abnormalities were detected in the plasma insulin itself in these patients. The data appear most compatible with an increased sensitivity of the pancreatic β-cell to a variety of stimuli in myotonic dystrophy.

In carriers (heterozygotes) of Duchenne muscular dystrophy, subtle histochemical abnormalities have been found, which differ from the more gross, and possibly factitious, ones recorded by other observers. The basic change consists of slightly atrophic fibers, scattered or in very small groups, of both histochemical types with the myofibrillar ATPase reaction; these fibers show nuclear activation and excessive amounts of cytoplasmic RNA basophilia and mitochondrial oxidative enzyme staining. Another histochemical enzymatic reaction which appears to be even more selective for staining these abnormal fibers, is being evaluated in numerous normal and disease-control biopsies.

It has been demonstrated in guinea pigs that only moderate external trauma to a muscle, produced by firm massage or brisk percussion with a reflex hammer, results in myopathic changes with inflammatory features in the underlying muscle. These results indicate caution is necessary in interpreting biopsies from human muscle which had received similar external trauma shortly before biopsy. In such instances the changes found may not necessarily be representive of histology throughout the body musculature.

In order to evaluate the role of ischemia in various muscle diseases and the resultant histochemical changes, rabbits have been subjected to various vascular occlusions, e.g. major arterial ligation, venous ligation, or embolization of distal arteroles. The affected muscles have been examined with histochemical techniques at varying times after occlusion. The data are being collated and compared to changes in various human muscle diseases.

In a patient with clinically typical "limb girdle dystrophy", amyloid has been found in the blood vessel walls of two muscle biopsies. This appears to represent the first example of an amyloid myopathy. The presence of amyloid only in the blood vessels raises the possibility that the muscle damage may be on an ischemic basis in that patient.

In a patient with abnormal muscle fatiguability and mild muscle swelling after exercise, an abnormality of marked hyperlacticacidemia following exercise was demonstrated. With histochemistry and electron-microscopy the major muscle fiber abnormality was excessive mitochondrial size and number, and bizzare internal structure. Biochemical assays of the muscle tissue are being explored to seek an underlying metabolic defect.

The muscle wasting in Cushing's disease (hypersecretion of the adrenal cortex) was found histochemically to be atrophy of muscle fibers, more of the type II's than the type I's, without associated changes of an ordinary myopathy (i.e. dissolution of

individual muscle fibers and endomysial connective tissue increase were not present). Measurement of intracellular electrolytes demonstrated a marked decrease of potassium.

A patient with the unusual combination of limb muscle myopathy (wherein the abnormal fibers contain morphologically abnormal mitochondria), extraocular muscle paralysis, corneal clouding, renal magnesium wasting, hypomagnesemic tetany, juxtaglomerular hyperplasia, and subacute necrotizing encephalomyelitis has been studied in depth.

Significance to Bio-Medical Research and the Program of the Institute: The myopathies represent a group of disorders rather similar clinically, the detailed differences of which have only begun to be worked out in the recent few years. One of the major methods for distinguishing these disorders is by histochemistry of the muscle biopsy. Other methods include electron-microscopy, biochemistry, and immunology. Hence, the distinguishing of various new forms of disease represent a step toward the overall understanding of this group of disorders. It is only when the detailed differences of the various forms of myopathy are analysed that the pathogenesis, etiology, and treatment can be understood. Identification of characteristic histochemical and biochemical defects in the myopathies provides leads to further explore the pathogenesis and possible treatment. The beneficial responses obtained in the therapy of polymyositis demonstrate additional regimens available in the management of such disorders.

Proposed Course of Project: The studies underway are part of a long-term project which will continue for several years.

Honors and Awards: None

Publications:

Engel, W.K., Vick, N.A., Glueck, C.J., and Levy, R.I.: A skeletal muscle disorder associated with intermittent symptoms and a possible defect of lipid metabolism. Trans. Amer. Neurol. Ass. In Press.

Gorden, P., Griggs, R.C., Nissley, S.P., Roth, J. and Engel, W.K.: Studies of plasma insulin in myotonic dystrophy. J. Clin. Endocr. In Press.

Hathaway, P.W., Dahl, D.S., and Engel, W.K.: Myopathic changes produced by local trauma. Arch. Neurol. In Press.

Serial No. NDS(I)-63 MN/OC 1037(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Microbiology Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
John W. Walsh, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.6
Professional:	1.1
Other:	0.5

Project Description:

Objectives: To study the growth characteristics, histochemical reactivities, immunologic properties, and electronmicroscopic details of skeletal muscle and motor neurons grown in tissue culture. The tissue is either normal chick, mouse or rat embryo or normal or abnormal biopsied human tissue, especially muscle.

Methods Employed: Standard tissue culture techniques are used as a basis, modified as necessary to obtain good growth from human cells. Applied to the cultured cells are other techniques being used in the Branch, e.g., histochemistry, electronmicroscopy, and immunology.

Patient Material: A portion of the muscle biopsy from certain patients with neuromuscular disease is taken for culture purposes.

Major Findings: This project was reactivated on a limited basis this past year due to delay in obtaining space and to personnel restrictions. The laboratory has been set-up and

reproducible cultures of chick embryo skeletal muscle and spinal cord are being obtained. The histochemical techniques for phosphorylase, myofibrillar ATPase, esterase, glycogen, and several oxidative enzymes have been applied successfully to the cultures.

The tissue culture facilities are also used on alternate days for short-term culture of human lymphocytes and thymocytes in studies of their immunologic properties, as described under the Immunology Project of this Branch.

Significance to Bio-Medical Research and the Program of the Institute: In addition to determining the histochemical properties of normal muscle and its growth characteristics in vitro, the studies of pathologic human muscle in vitro will be used to seek disturbed growth patterns and, more particularly, to determine ways of improving deficient growth. Agents found to improve deficient growth in vitro will then be tested for their usefulness in the patient with muscle disease to promote growth of abnormal muscle or to retard its degeneration. As a foundation for understanding phenomena in human muscle, chick embryo material has been studied. Study of motor neurons grown in vitro is part of a multilateral approach to diseases which preferentially involve human motor neurons, such as amyotrophic lateral sclerosis.

Proposed Course of Project: Further studies of normal and pathologic human muscle in vitro will be done. Definitive studies, starting with histochemistry, will be applied to the cultured motor neurons. Electron-microscopy of the cultured muscle and nerve cells is planned.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/OC 1039(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Amyotrophic Lateral Sclerosis (ALS) and Other Lower Motor Neuron Diseases

Previous Serial Number: Same

Principal Investigator: W. King Engel, M.D.

Other Investigators:
Jon D. Dorman, M.D.
Robert I. Levy, M.D.
Frederic Q. Vroom, M.D.
Thomas A. Waldmann, M.D.
Donald S. Schalch, M.D.
David M. Fried, M.D.
Darryl C. DeVivo, M.D.
Nicholas A. Vick, M.D.
Michel Fardeau, M.D.
John A. Morgan-Hughes, M.D.
John L. Sever, M.D.
Jerome S. Resnick, M.D.
Clarence J. Gibbs, Jr., M.D.

Cooperating Units:
Molecular Disease Branch, NHI
Medicine Branch, NCI
University of Rochester School of Medicine
Rehabilitation Department, CC
Collaborative and Field Research, NINDS
Perinatal Research Branch, NINDS

Man Years

Total:	2.6
Professional:	2.5
Other:	0.1

Project Description:

Objectives: In ALS and other diseases affecting the lower motor neurons, we are seeking (a) more precise morphologic and chemical definition of the abnormalities; (b) separation of each disorder into more distinct, and often new, subforms; (c) specific

or symptomatic therapeutic response; (d) new methods of analysing the abnormalities and (e) animal models of the human pathophysiologic states.

Methods Employed: The techniques of histochemistry, biochemistry, autoradiography, electron-microscopy, immunology and electromyography were as detailed in other project reports. Additional methods were as follows. Double-blind therapeutic trials, the efficacy of which is judged by clinical testing, functional evaluation, and serial quantitative evaluation of muscle function using an apparatus designed by us. Carbohydrate metabolism studies - glucose, tolbutamide, and insulin tolerance tests; assay of plasma insulin (with U. Rochester). Lipoprotein studies (with NHI). Analysis of motor areas of brain and spinal cord by histochemistry and electron-microscopy. Biochemical assays of serum fatty acids (with NHI) and turnover of serum protein fractions (with NCI). Search for virus by electron-microscopy, and by inoculation of tissue cultures and animals (with PRB and C&FR).

Patient Material: Medical Neurology Branch patients.

Major Findings:

MOTOR NEURON DISORDERS

The intolerance of oral glucose in about 30% of ALS patients was reported to be not disease specific (in contrast to the reports of others), being present in essentially the same percent of four groups of patients with other neuromuscular diseases (childhood muscular dystrophy, myotonic dystrophy, late-onset dystrophy, and chronic peripheral neuropathy). Studies of tolbutamide tolerance, insulin tolerance, and assay of plasma insulin (with U. Rochester) indicate (a) no consistent abnormality of pancreatic endocrine function in ALS, (b) those patients who have intolerance to oral glucose usually do not have concomitant hyposecretion of insulin, (c) only a minority (10%) of the 10 ALS patients (out of 22 studied) with definite or borderline insulin hyposecretion (demonstrated in a single test) have concomitant definite oral glucose intolerance. It is possible that different factors may be responsible for glucose intolerance of individuals within the ALS group - but if they do have a common denominator, results of the several tests fit best with an hypothesis of reduced glucose receptor space due to decreased functional muscle mass. Virtually none of 124 ALS patients had an elevated fasting blood glucose level, glycosuria or other signs of diabetes mellitus. Treatment of some who had oral glucose intolerance by dietary regulation and tolbutamide did not result in neurologic benefit. There is thus no evidence that pancreatic endocrine malfunction is a necessary step in the pathogenesis of ALS.

In amyotrophic lateral sclerosis (ALS) it was reported that no abnormality of the following metabolic processes could be demonstrated: synthesis and catabolism of IgA and IgG, catabolism of caeruloplasmin and catabolism of albumin, each with isotope-labeled protein (with NCI); plasma lipoprotein content, by paper electrophoresis; plasma fatty acids, and specifically phytanic acid, by gas-liquid chromatography (with NHI); serum arginine content; aminoacid excretion, by aminoacid analysis of urine.

Using the apparatus we have designed and built for quantitating muscle strength (with Physical Medicine, CC) and shown to give reproducible results in normal controls and patients as a supplement to clinical examination, it was demonstrated that vitamin E plus pancreatic extract was ineffective in altering the progressive course of ALS in 12 patients, contrary to the reports of others. Furthermore, lipoic acid, adamantine (an antiviral agent), prednisone, guanidine, and intravenous plasma were also reported to be of no therapeutic value in ALS on the basis of double-blind trials.

Two sporadic cases of infantile spinal muscular atrophy (ISMA) were reported to contain eosinophilic nuclear inclusion bodies in motor neurons, raising the question of a viral factor.

Attempts to transmit an infectious agent to tissue cultures and intracerebrally to mammals are underway, using material from patients with ALS, infantile spinal muscular atrophy (ISMA), inclusion body encephalitis and certain dementias (with NINDS-CF). To date after several years in some instances, the ALS and ISMA material is negative.

The occurrence of infantile and juvenile forms of chronic spinal muscular atrophy in the same families was put forth as further evidence that these two forms are different manifestations of the same disease. In fact, it was noted that the majority of cases of "juvenile" spinal muscular atrophy actually have symptoms dating back to infancy.

Analysis of brain and spinal cord material from ALS and ISMA patients by 10 histochemical reactions disclosed no specific defect in either disease. Prominent astrocytic response in cerebral white matter was demonstrated by oxidative enzyme reactions. Motor neurons and subpial glia showed prominent phosphorylase activity (and slight activity in reactive astrocytes of white matter) in these patients and in other disease states, indicating a brisk capacity for anerobic glycolysis in these cell populations. Electron-microscopic studies of biopsied ALS cortex showed abnormal structures within neuronal nuclei (see Electron-Microscopy Project of this Branch).

The histochemical changes of muscle in ALS was compared and contrasted with changes in other motor neuron diseases and peripheral neuropathies. The earliest lesion was atrophy of individual muscle fibers, not groups as generally taught - this suggests that muscle fibers in a given motor unit are scattered rather than grouped as usually stated.

In one family, the combination of type II hyperlipoproteinemia (hyperbetalipoproteinemia) and chronic progressive muscular atrophy affected 3 brothers but not their sister (with NHI). A possible relationship between the metabolic disorder and the motor neuron degeneration is being sought.

The first family with neurologic disease, acanthocytosis of the erythrocytes, and normal plasma β-lipoprotein was found in this Branch (with NHI and NIAMD). Subsequently another family was reported from Kentucky. We have recently found a third family. The clinical neurologic findings are now sufficiently characteristic that the syndrome can at least be suspected clinically. A common denominator between the erythrocyte abnormality and the neurologic disease is being sought. These families demonstrate that acanthocytosis associated with neurologic disease is not invariably accompanied by abetalipoproteinemia, as was earlier thought from the study of Bassin-Kornsweig disease.

An extensive survey of "Motor Neuron Disorders" was written as a chapter for a neurology text conceived and planned by the late Dr. G. Milton Shy to combine in each chapter a review of the current clinical status of the neurologic disease with the clinical investigative and basic research aspects of the particular system affected. That correlation was attempted in the Motor Neuron Disorder chapter.

One possible cause of ALS is a chronic viral infection. Therefore, subacute sclerosing panencephalitis (SSPE), is being studied as a possible model, since the likelihood of its being a chronic measles encephalitis is rather strong. Although (SSPE) is generally considered inexorably progressive to death, we documented a clinically typical case, with the expected very high measles antibody levels in blood and spinal fluid (with PRB, NINDS), who showed striking spontaneous clinical improvement (without change of antibody levels). This remission indicates caution is necessary in interpreting the effect of drug treatment in SSPE when controlled studies with an adequate number of patients are not done. Brain biopsy material from this patient inoculated into animals has failed to provoke disease in them (with C&FR, NINDS).

PERIPHERAL NEUROPATHIES

In a patient with idiopathic recurrent neuropathy associated with high CSF protein, two new aspects to the use of adrenocorticosteroids (oral prednisone) have been found. The patient, who had been quadriplegic 11 months prior to treatment, did not begin to respond until after the medication had been given daily for 4 months. The response was then gradual to the point where the patient was walking by herself, and improvement is still occurring, 17 months after prednisone was started. Moreover, for the past 5 months prednisone has been given on a every-other-day program, resulting in reduction of "cushingoid" side effects and continued improvement of the patient.

In Refsum's disease (with hyperphytanicacidemia), the therapeutic dietary attempts (with NHI) to circumvent the biochemical defect and the electron-microscopy of the hypertrophic neuropathy are described in our other projects (Biochemistry; Electron-Microscopy).

In the study of single teased nerve fibers from peripheral nerves, a method of staining with DPNH, DPNH-linked lactate, and DPN-linked malate dehydrogenases was developed. It showed high activity in schwann cell cytoplasm of the region around the schwann nucleus and of the delicate schwann cell processes in the region of ranvier's node. The myelin sheath was unstained, and the axon had slight activity. When these techniques were used to study the histochemical changes in single teased axons following experimental nerve crush in the intact guinea pig and cat, it was found that darkly staining material accumulated both proximally and distally to the crush site. In single fibers, a steady retraction of the axon, slipping within the myelin sheath away from the crush site, was plotted over the first 24 hours. The retracted axons formed coils, spirals, and fusiform swellings. The demonstration of axonal retraction suggested elastic properties of axons and indicated that due consideration must be given to the phenomenon of axonal retraction before accumulated enzyme-rich (or silver-stained) material can be attributed to dammed-up axoplasmic flow.

The Lambert-Eaton facilitating myasthenic syndrome was described in a 27 year old lady who has had for 10 years weakness that responded to chronic corticosteroid administration. No evidence of a neoplasm can be found. The physiologic and clinical defect responded to guanidine hydrochloride, which facilitates quantum release of acetylcholine from the motor neuron tip and is generally effective in more typical cases of the myasthenic facilitating syndrome. The possibility that this patient has an abnormality in the terminal portions of her motor neurons that

presented initially as progressive neuropathy is being further studied.

An unusual instance of an ordinary myasthenic syndrome (clinical fatiguability responsive to anticholinesterases and worsened by curare) was demonstrated in a patient with previous poliomyelitis. Although possibly the two disorders are occuring together by chance, more interesting is the possibility that previous or underlying motor neuropathy can in some instances, such as this, be manifest by a chronic functional defect in distal motor nerve function, i.e. myasthenic reaction.

A new method for recording motor axon potentials in man has been found.

Significance to Bio-Medical Research and the Program of the Institute: New details have been accumulated on the morphologic and biochemical substrata of ALS and other diseases of the lower motor neuron. More accurate evaluation of therapeutic trials are facilitated by (a) development of an instrument for quantitative muscle testing in patients and (b) more complete knowledge of variations in the natural course of the forms of motor neuron diseases. If dietary treatment of hyperphytanicacidemia (Refsum's disease) continues to be beneficial, another form of neurologic/ metabolic disease will have been benefited by circumvention of the known metabolic defect. New aspects of the beneficial use of prednisone in chronic neuropathy indicate additional patients should be tried on the drug on a prolonged program, possibly every-other-day, even if they have been severely paralysed months or longer. New histochemical methods of studying peripheral nerves have been developed and may now be applied to human diseases of peripheral nerves and experimental animal neuropathies.

Proposed Course of Project: To more fully develop studies underway in the hope that identification of metabolic, immune, or infectious etiologies will lead to a means of treating and preventing these disorders. To apply tissue culture techniques to these problems when space becomes available. To undertake additional therapeutic trials.

Honors and Awards: None

Publications:

Collis, W.J., and Engel, W.K.: Glucose metabolism in five neuromuscular disorders. Neurology 18: 915-925, 1968.

Engel, W.K.: Motor neuron histochemistry in ALS and infantile spinal muscular atrophy. In Norris, F.H., Jr. and Kurland,

L.T. (Eds.): Motor Neuron Diseases. New York, Grune and Stratton, 1969, pp. 218-234.

Engel, W.K., and Brooke, M.H.: Muscle biopsy in ALS and other motor neuron diseases. In Norris, F.H., Jr., and Kurland, L.T. (Eds.): Motor Neuron Diseases. New York, Grune and Stratton, 1969, pp. 154-159.

Engel, W.K., Hogenhuis, L.A.H., Collis, W.J., Schalch, D.S., Barlow, M.H., Gold, G.N., and Dorman, J.D.: Metabolic studies and therapeutic trials in amyotrophic lateral sclerosis. In Norris, F.H., Jr., and Kurland, L.T. (Eds.): Motor Neuron Diseases. New York, Grune and Stratton, 1969, pp. 199-213.

Morgan-Hughes, J.A., and Engel, W.K.: Histochemical patterns in single peripheral nerve fibers. Arch. Neurol. 19: 613-617, 1968.

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Resnick, J.S., Engel, W.K., and Sever, J.: Subacute sclerosing panencephalitis: Spontaneous improvement in a patient with elevated measles antibody in blood and spinal fluid. New Eng. J. Med. 279: 126-129, 1968.

Vroom, F.Q., and Engel, W.K.: Non-neoplastic steroid-responsive Lambert-Eaton myasthenic syndrome. Neurology 19: 281, 1969.

Dorman, J.D., Engel, W.K., and Fried, D.M.: Therapeutic trials in amyotrophic lateral sclerosis. Lack of benefit with pancreatic enzymes and vitamin E in 12 patients. JAMA. In Press.

Engel, W.K.: Motor Neuron Disorders. In Shy, G.M., Goldensohn, E.S., and Appel, S.H. (Eds.): The Cellular and Molecular Basis of Neurologic Disease. Philadelphia, Lea and Febiger. In Press.

Tobin, W.E.: A method for recording motor axon potential in man. J. Appl. Physiol. In Press.

Serial No. NDS(I)-65 MN/OC 1189(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Episodic Weakness

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
Robert C. Griggs, M.D.

Other Investigators: Jerome S. Resnick, M.D.
Pieter R.A. Kark, M.D.
Frederick C. Bartter, M.D.

Cooperating Units: Endocrinology Branch, NHI

Man Years

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description:

Objectives: To define more clearly and treat those disorders affecting the neuromuscular apparatus which present primarily with episodic weakness or paralysis. Attention is directed toward those conditions in which evidence suggests that the main site of intermittent dysfunction is somewhere within the following portions of the muscle fiber: sarcolemmal membrane; and sarcolemmal-T system-sarcoplasmic reticulum-myofibrillar complex (excitation-contraction coupling mechanism). Studies are done with agents which are either provocative or therapeutic with respect to periodic paralysis syndromes, with a view to obtaining more information regarding pertinent metabolic pathways and methods of treatment.

Methods Employed: The techniques of clinical investigation (including electromyography and clinical biochemistry), muscle biopsy with samples for histochemical analysis, electron-microscopy, and biochemical assays of tissue were delineated in other MNB projects. Provocative loading tests and therapeutic trials to raise or lower potassium or sodium are used.

Acetazolamide, triamterene, and ammonium chloride were administered as possible prophylactic agents for hypokalemic periodic paralysis. Electrolyte content and balance were evaluated on and off therapy.

Patient Material: Patients of all ages are admitted to the Medical Neurology Branch for this project if they have: intermittent muscular weakness associated with familial periodic paralysis, hypo- or hyperkalemic; isolated examples of periodic paralysis with potassium disturbance; thyrotoxic periodic paralysis; paramyotonia congenita; or myotonia congenita. (Patients with myasthenia gravis are part of another Medical Neurology Branch Project.)

Major Findings: Acetazolamide (Diamox), introduced by us last year as a new prophylaxis in hypokalemic periodic paralysis, continues to be very effective with no significant side effects in 2 patients followed an additional year on therapy (total of 3 years each) and in 10 additional patients. Nine of 12 patients with hypokalemic periodic paralysis, both sporadic and familial, have responded with dramatic decrease in attack frequency - 6 who had had almost daily attacks despite large doses of prophylactic potassium have had no attacks on acetazolamide. The study was single-blind, placebo-controlled, with frequent cross-overs. Seven of the 12 had moderate or severe persistent proximal weakness between attacks even on conventional therapy - on acetazolamide 5 improved, 3 of these having returned to normal strength.

The mechanism of acetazolamide benefit in this disorder is unknown. Skeletal muscle is thought not to contain carbonic anhydrase. Studies in these patients indicated acetazolamide did not significantly alter serum electrolytes, blood pH, or thyroid function. Balance studies showed the drug to cause an initial increase of potassium excretion but no long-term change of total body potassium despite persistent therapeutic effect. Similarily, despite slightly increased initial excretion of sodium, no eventual effect on total exchangeable body sodium was found. Another diuretic, triamterene, which produced a greater natruresis, had no therapeutic effect. Ammonium chloride was without therapeutic effect.

A thyrotoxic form of hypokalemic paralysis has been cured with antithyroid treatment and the concomitant metabolic changes investigated. Reserpine treatment alone reduced the pulse and tremor to normal but did not prevent attacks and interim weakness, whereas propylthiouracil did cure the paretic attacks and the constant between-attack weakness. An intravenous glucose-and-insulin provocation prior to antithyroid therapy produced striking hypokalemia and paralysis, but after antithyroid treatment an identical challenge failed to do so. The aldosterone secretion

and metabolic clearance rates were strikingly elevated, according to a single observation, before any treatment of the thyrotoxicosis; after the euthyroid state was achieved the secretion rate was normal and the metabolic clearance rate actually low (with NHI). Hence the plasma concentration was the same before and after.

Significance to Bio-Medical Research and the Program of the Institute: Our introduction of acetazolamide in the treatment of hypokalemic periodic paralysis has provided the most effective prophylaxis available in this disorder. It remains reliably effective and safe over a long period of time (more than 3 years to date). It benefits about 75% of the patients tested. This new form of prophylaxis provides new avenues for investigation of the basic pathophysiology in this disease. The results in thyrotoxic hypokalemic periodic paralysis suggest epinepherin does not play a role in the weakness, but a secondary hyperaldosteronism has not been ruled out.

Proposed Course of Project: To explore in more detail, with patients and animals, the mechanism of action of acetazolamide prophylaxis in hypokalemic periodic paralysis and the pathogenesis of the disease itself. To seek even more effective therapeutic agents. To investigate the pathogenesis of thyrotoxic periodic paralysis.

Honors and Awards: None

Publications:

Griggs, R.C., Engel, W.K., and Resnick, J.S.: Acetazolamide therapy in hypokalemic periodic paralysis: prevention of attacks and improvement of persistent weakness. Neurology 19: 281-282, 1969.

Dorman, J.D., Resnick, J.S., and Engel, W.K.: Thyrotoxic periodic paralysis - A case study. Amer. J. Med. In Press.

Serial No. NDS(I)-65 MN/OC 1190(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Myasthenia Gravis

Previous Serial Number: Same

Principal Investigators: Dale E. McFarlin, M.D.
W. King Engel, M.D.
Gregory Walsh, M.D.

Other Investigators: John S. Johnson, M.D.

Cooperating Units: Laboratory of Clinical Investigation, NIAID

Man Years

Total:	1.7
Professional:	1.2
Other:	0.5

Project Description:

Objectives: To apply histochemical, pharmacologic, electrophysiologic and immunologic techniques to investigate the pathogenesis of myasthenia gravis. Immunologic studies were included because: 1. Immunologic abnormalities have recently been demonstrated in the serum of myasthenic patients. 2. Thymic pathology in patients with myasthenia is well known, and it is now felt that this gland has major immunologic functions.

Methods Employed: Sera from patients with myasthenia gravis were studied by a variety of immunologic techniques, including agar diffusion, immunoelectrophoresis, latex agglutination, gel filtration, ion exchange chromotography, and protolytic digestion of proteins. Lymphocytes and thymocytes were grown in short-term culture and their responsiveness to common cellular stimulants was measured by incorporation of tritiated thymidine in DNA. Specimens of muscle, and in some cases thymus and thyroid, were studied using immunofluorescent, histochemical and routine histological techniques. The abnormalities were evaluated before and after thymectomy. Clinical studies including prostigmine and

curare challenging tests, were done on patients with myasthenia gravis, on a group of patients with myasthenic syndrome in association with clinical entities with established denervation, and on a number of patients with a variety of other neuromuscular diseases.

Patient Material: Sera, muscle, thymus and other tissue were obtained from Medical Neurology Branch patients.

Major Findings: The immunoglobulins responsible for antinuclear factor and antimuscle factor in serum from myasthenic patients were characterized. Antinuclear factor was found to occur in IgG, IgM and IgA and in some patients was found in all three classes of immunoglobulin. In contrast, antimuscle factor was found to occur only in IgG in both myasthenic patients and nonmyasthenic patients with thymoma. This study demonstrated that these two forms of reactivity behave as separate entities.

The portion of the IgG molecule responsible for antimuscle factor was ascertained and was shown to reside in the FAB fragment but not the FAC fragment.

Treatment of one myasthenic patient with ACTH produced striking improvement. During this treatment no change in the absolute lymphocyte count or titer of AMF was seen.

Thymocytes from one patient with myasthenia gravis responded to phytohemagglutinin and poke weed mitogen in a fashion similar to that of peripheral blood lymphocytes, unlike normal thymocytes.

Significance to Bio-Medical Research and the Program of the Institute: The demonstration that the antimuscle factor in patients with myasthenia gravis does not differ immunochemically from the antimuscle factor in patients with thymoma and no myasthenia gravis supports our suspicion that this agent per se is not responsible for the neuromuscle block in this disease. The demonstration that antimuscle factor is due to the FAB portion of the IgG molecule supports the concept that this serological reactivity is an antibody and thus develops as part of an immunological process. The observation that antimuscle factor and antinuclear factor behave as separate serological entities raises the possibility of a third as yet undescribed serological abnormality being the agent responsible for the major physiologic defect in this condition.

Finding cells in the thymus of a patient with myasthenia gravis which respond to stimulants supports the notion that reactive cells are present in thymus of patients with this disease. This is consistent with the concept of a thymitis and

is in contrast to normal thymus in which the thymocytes are non-responsive. The response of myasthenic patients to ACTH indicates that much of the abnormality is not irreversible and supports the idea that improvement can result even in the most severely affected and advanced stages of this disease.

Proposed Course of Project: In selected patients, specific aspects of this disease will be investigated: post-thymectomy state, neonatal myasthenia, mechanism of ACTH treatment, and newer forms of therapy. Techniques involving the study of cellular immunity are to be added to the investigative program when new space and personnel are available for a tissue culture laboratory, which should facilitate characterization of the thymic abnormality in myasthenia gravis.

Honors and Awards: None

Publications:

McFarlin, D.E.: Serological abnormalities in myasthenia gravis. Neurology 18: 274, 1968.

McFarlin, D.E., Johnson, J.S., and Seymour, W.F.K.: Anti-muscle factor and antinuclear factor in patients with myasthenia gravis. I. Heavy chain determinants. J. Immunol. 101: 104-110, 1968.

Griggs, R.C., McFarlin, D.E., and Engel, W.K.: Severe occult juvenile myasthenia gravis responsive to long-term corticosteroid therapy. Trans. Amer. Neurol. Ass. 93: 216-218, 1968.

McFarlin, D.E.: Myasthenia gravis. In Samter, M. (Ed.): Immunological Diseases. Boston, Little, Brown and Company. In Press.

Serial No. NDS(I)-65 MN/OC 1191(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Immunological Abnormalities of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: Dale E. McFarlin, M.D.

Other Investigators:
Thomas A. Waldmann, M.D.
Warren Strober, M.D.
Joost Oppenheim, M.D.
John S. Johnson, M.D.
Darryl C. DeVivo, M.D.
Nicholas Vick, M.D.
John L. Sever, M.D.

Cooperating Units:
Metabolism Branch, NCI
Medicine Branch, NCI
Human Genetics Branch, NIDR
Laboratory of Clinical Investigation, NIAID
Perinatal Research Branch, NINDS

Man Years

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

Objectives: To apply immunological techniques to the study of diseases affecting the central nervous system and neuromuscular system.

Methods Employed: Standard immunochemical techniques were employed. In addition, preliminary use of tissue culture techniques was begun, especially to study lymphocyte transformation.

Patient Material: Specimens of peripheral blood, bone marrow, muscle, peripheral nerve, thymus and other tissue was obtained from patients with neurological disease admitted to the Medical Neurology Branch.

Major Findings: 1. In ataxia-telangiectasia the following were found: (a) In a total of 12 patients a defect of IgA synthesis has been confirmed. (b) Low molecular weight IgM has been documented in the serum of patients lacking IgA but has not been found in the saliva of such patients. (c) A defect in the cellular response to common lymphocytes stimulants has been documented. (d) The defect in cellular response has been shown to be in part related to a serological factor which inhibits the response of normal cells. This defect can be overcome by suspending cells from ataxia-telangiectasia patients in normal plasma and by also increasing the concentration of stimulants employed. (e) Defective production of circulating antibody was seen. (f) Approximately 60% of patients with ataxia-telangiectasia have documented hepatic dysfunction. To date the mechanism of this has not been elucidated specifically, no evidence of a hepatitis secondary to the immunological deficit has been documented. (g) Aminoaciduria has been seen in 4 patients with ataxia-telangiectasia, all of whom have hepatic dysfunction. (h) Cultures of tissue obtained at autopsy, urine sediment and WBC have been negative for non-bacterial agents. (i) Electron microscopy of lymphocytes from three cases and muscle from another showed no abnormalities of basement membranes.

2. Low molecular weight immunoglobulin M has been the subject of extensive study. This protein was demonstrated for the first time in the spinal fluid of a patient with recurrent encephalopathy. Metabolic studies in this patient were consistent with a non-neoplastic immunological response and it appeared that this protein did not result from the catabolism of the high molecular weight IgM. Low molecular weight IgM was also demonstrated as a monotypic variety in a patient with amyloidosis and was found in approximately 70% of the patients with ataxia-telangiectasia and in some patients with myasthenia gravis.

3. Immunological evaluation of a patient with subacute sclerosing panencephalitis with high titers of measles antibody revealed a normal response to blood group antigen, normal responses to skin antigens, and normal lymphocyte transformation to phytohemagglutinin.

4. Specimens collected from every Medical Neurology Branch patient and stored in the serum and spinal fluid bank have proven to be valuable in the study of a number of entities of possible viral and/or autoimmune etiology.

Significance to Bio-Medical Research and the Program of the Institute: The underlying defect in ataxia-telangiectasia remains undelineated; however, the above data suggest that the immunological defect is in part related to a serological factor which exerts some degree of inhibition on lymphocyte function.

The demonstration of hepatic dysfunction in over 50% of these patients raises the possibility that the underlying defect may indeed be of hepatic origin. The aminoaciduria seen in approximately 15-20% of ataxia-telangiectasia patients is likely secondary to hepatic dysfunction. The combination of abnormal response to lymphocyte stimulants and an insulin resistant diabetic state raises the question of an underlying membrane defect. However, electron microscopic studies of nerve and muscle from one patient failed to disclose any morphological abnormalities of the membranes of these tissues.

Elucidation of the defects responsible for abnormalities in ataxia-telangiectasia may lead to learning the mechanism responsible for the nervous system pathology. Whether the underlying immunological defect present in ataxia-telangiectasia predisposes to an infection ultimately responsible for the central nervous system disease remains to be seen. Viral studies on our patients have thus far been negative. The mechanism in this disease, when elucidated, may have direct bearing on the pathogenesis of multiple sclerosis and other acquired diseases of the brain.

Low molecular weight IgM may be of considerable importance in infectious processes involving the central nervous systems. Some antigens elicit an antibody response in only one antibody class. When the antibody class is IgM the resulting antibody would have some difficulty entering the central nervous system if it were the usual high molecular weight gamma M which has a high molecular weight and high diffusion coefficient. However, the low molecular weight gamma M under study is smaller in size and has a lower diffusion coefficient and thus would be expected to enter the CNS more readily.

Proposed Course of Project: The immunological defects in ataxia-telangiectasia are to be categorized more fully with the addition of the latest immunological techniques. Additional studies of cellular immunity in a number of neurological conditions are planned to be started when additional space, necessary equipment and personnel become available.

Metabolic studies of the origin and passage of low molecular weight IgM are planned.

Honors and Awards: None

Publications:

McFarlin, D.E., and Griggs, R.C.: Treatment of inflammatory myopathies with azathioprine. Trans. Amer. Neurol. Ass. 93: 244-246, 1968.

Strober, W., Wochner, R.D., Barlow, M.H., McFarlin, D.E., and Waldmann, T.A.: Immunoglobulin metabolism in ataxia telangiectasia. J. Clin. Invest. 47: 1905-1915, 1968.

Griggs, R.C., Strober, W., and McFarlin, D.E.: Studies of low molecular weight γM (LMW-γM) in a patient with systemic lupus erythematosis (SLE). Clin. Res. 16: 545, 1968.

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Serial No. NDS(I)-65 MN/OC 1192(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electron-Microscopic Studies of Skeletal Muscle and Neurons

Previous Serial Number: Same

Principal Investigators: W. King Engel, M.D.
William H. Olson, M.D.
Nicholas A. Vick, M.D.

Other Investigators: Michel Fardeau, M.D.
Jerome S. Resnick, M.D.

Cooperating Units: None

Man Years

Total:	2.5
Professional:	2.0
Other:	0.5

Project Description:

Objectives: To study the early subcellular changes of human muscle in diseases that are confined to muscle alone, as well as in conditions which produce secondary alterations in muscle, such as denervating and metabolic diseases. To study subcellular changes of human neurons in certain diseases.

Methods Employed: A portion of biopsied muscle is tied to a glass rod to maintain resting length and immediately fixed in cold buffered glutaraldehyde or osmium tetroxide for eventual araldite embedding. Other fixative combinations and buffers have also been experimented with. Various electron-microscopic (EM) staining procedures are used. Adjacent biopsy material is immediately frozen for cryostat sections and incubated in various histochemical media, as described in the Histochemistry Project. Histochemically stained cryostat sections are ready within half an hour after the biopsy and are used as a guide to determine which of the biopsies contain light microscopic features of special interest and thereby serve as a guide for choosing material appropriate for electron-microscopic study. Biopsy

specimens of human nerve or brain tissue are fixed and embedded for electron-microscopy and are similarly monitored by rapid enzyme histochemistry of adjacent tissue.

Patient Material: Patients with various myopathies and neurogenic muscular weakness are the major sources of material. When available, tissue from asymptomatic carriers of progressive genetic myopathies is taken for study. Muscle biopsies and nerve biopsies are the major tissues examined. Occasional brain biopsies are obtained from patients with certain forms of progressive degenerative disease. Other abnormal tissues from neurologic patients are infrequently examined.

Major Findings: In rod (nemaline) myopathy, identified from this Branch in 1961, there is accumulation in the patient's muscle fibers of tiny, rod-shaped particles containing a periodicity of 145-170Å; the rods are centered on and appear to arise from the Z-line. An experimental source of morphologically identical rods has now been developed, by virtue of the EM finding that rods induced in cat skeletal muscle by tenotomy have the same periodicity and Z-band centering.

In Refsum's disease (atactica hereditaria polyneuritiformis), which has a concomitant hyperphytanicacidemia, the neuropathy was demonstrated to be primarily a defect of Schwann cells, these cells showing (a) marked hypertrophy, and (b) very distinctive "crystalline-like" inclusions in mitochondria.

Details of mitochondrial abnormalities - enlargement, amorphous lipid-like inclusions, organized box-car-like inclusions between the inner and outer membrane, and contorted cristae - have been elucidated in three unusual kinds of muscle disease.

Accumulation of lipid droplets in otherwise normal muscle fibers (including normal mitochondria) has been demonstrated in a new myopathy associated with defective ketone-body production (see Myopathy Project).

What were initially described from this Branch on the basis of light microscopic histochemical studies as "mitochondrial aggregates" have now been found by EM to be "tubular aggregates", apparently collections of proliferated sarcoplasmic reticulum.

The ultrastructure of muscle fibers in the new disease "type I fiber hypotrophy with internal nuclei" reported from this Branch have been demonstrated.

An EM search for viral particles in polymyositis and other sporadic neuromuscular diseases has been initiated, in an attempt

to confirm one report by another group. To date, none has been found.

Ultrastructural analysis of blood vessels in patients with collagen-vascular-disease forms of polymyositis (including dermatomyositis) so far fails to confirm a report from another laboratory that thickening of the intramuscular capillary basement membrane is characteristic of this disease group.

"Ragged red fibers", identified histochemically (rich in neutral lipid and mitochondrial oxidative enzymes) in clinically normal skeletal muscle as the most common lesion in patients with slowly progressive external ophthalmoplegia, have been described electron-microscopically - they contain excessive amounts of lipid droplets in the sarcoplasm and groups of abnormal mitochondria.

Abnormal structures within neuron nuclei have been identified in patients with sporadic and hereditary ALS.

Virus-induced brain tumors from dogs are being looked at to compare their ultrastructure and possible viral particle content with "spontaneously" arising (? viral) brain tumors of humans.

Significance to Bio-Medical Research and the Program of the Institute: Analysis of the changes in ultrastructure of the neuromuscular disorders is in its infancy. Identification of new forms of disease occur rapidly in this stage. However one must be careful not to name a condition on the basis of careful though limited EM or histochemical studies. Therefore, a broad experience with as many well-studied cases as possible is necessary, as is broad correlation of EM with histochemistry. It appears that certain morphologic changes can indicate suspected metabolic defects, not necessarily "primary", as in contractile protein defects in rod myopathy and sarcoplasmic reticulum in "tubular aggregates". Other ultrastructural changes, e.g., accumulation of lipid in otherwise normal fibers, provide strong clues as to which metabolic pathways are likely to be the site of a presumed enzyme defect. These ultrastructural changes will help guide formulation of pathogenesis and treatment of the neuromuscular and neurologic disorders. Experimental production of identical defects, e.g., rods in animals, confirmed by EM, provides a new tool for analysing these changes. Similar expectations pertain to the other human neuromuscular disorders being studied, as well as the neuronal abnormalities. The chemical substance composing the "crystalline" inclusions in Refsum's disease may provide further clues of the pathogenesis of this disease.

Proposed Course of Project: Further studies that combine histochemical, immunologic, autoradiographic and tissue culture techniques with electron-microscopy applied to human neurologic diseases and animal models have been planned, so as to supplement the morphologic approach with pertinent studies of cellular dynamics.

Honors and Awards: None

Publications:

Fardeau, M., and Engel, W.K.: Ultrastructural study of a peripheral nerve biopsy in Refsum's disease. J. Neuropath. Exp. Neurol. 28: 278-294, 1969.

Resnick, J.S., Engel, W.K., and Nelson, P.G.: Changes in the Z disk of skeletal muscle induced by tenotomy. Neurology 18: 737-740, 1968.

Serial No. NDS(I)-65 MN/OC 1193(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Radioautography Applied to the Study of Neurologic Disease

Previous Serial Number: Same

Principal Investigators: David E. Pleasure, M.D.
W. King Engel, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.9
Professional:	1.4
Other:	0.5

Project Description:

Objectives: To apply radioautographic techniques to the study of alterations in protein metabolism of motor and sensory neurons in experimentally induced neuropathies.

Methods Employed: Radioautography was employed using the excellent resolution afforded by the low energy β-radiation of tritiated amino acids. Labelled proteins were located in the central and peripheral nervous systems at intervals following administration of tritiated leucine in normal and neuropathic cats. Preliminary experiments on the isolation and characterization of these proteins were carried out using centrifugation and column chromatography.

Patient Material: Patient material was not utilized.

Major Findings: Study of the pattern of distribution of radioactivity in dorsal and ventral nerve roots after administration of tritiated leucine indicates that in normal cats there is a flow of labelled proteins from site of synthesis in neuronal cell bodies along axonal processes at approximately 1 1/2mm/day.

This flow is interrupted in cats made neuropathic by acrylamide but remains normal in those with triorthocresyl phosphate induced neuropathy.

Preliminary biochemical studies indicate that the labelled substance flowing along axons has a molecular weight greater than 1500 and is rather strongly bound to DEAE-cellulose at pH 7.0.

Significance to Bio-Medical Research and the Program of the Institute: The findings indicate the apparently selective axonal destruction induced by one toxin (acrylamide) may be due to a failure of synthesis by the neuron cell body of proteins essential for axonal integrity; consequently certain inherited and metabolic diseases of the human nervous system with similar selective axonal destruction may have the same pathophysiologic defect.

Proposed Course of Project: Planned extensions of this study of axonal flow include the separation and characterization, by biochemical methods, of the proteins that are synthesized in the cell bodies of neurons and destined for export to their axonal processes.

Honors and Awards: None

Publications:

Pleasure, D.E., Mishler, K.C., and Engel, W.K.: Axonal flow in neuropathies. Science. In Press.

Serial No. NDS(I)-69 MN/OC 1728(c)
1. Medical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Brain Tumor Induction with Schmidt-Ruppin Rous Sarcoma Virus (SR-RSV)

Previous Serial Number: None

Principal Investigators: Darrel D. Bigner, M.D.
John P. Kvedar, A.B.

Other Investigators: W. King Engel, M.D.
Nicholas A. Vick, M.D.
Dale E. McFarlin, M.D.
W. Ray Bryan, Ph.D.

Cooperating Units: Viral Oncology Branch, NCI

Man Years

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

Objectives: (1) To develop a brain tumor animal model in which to study the pathogenesis and better methods of diagnosis of brain tumors; (2) to develop the most sensitive animal system in which to attempt recovery of oncogenic viruses from human brain tumors; (3) to improve methods of virus extraction from SR-Rous sarcoma virus (SR-RSV) tumors.

Methods Employed: a. Assay of SR-RSV, an RNA tumor-inducing virus, on the chorioallantoic membrane of chicken eggs.

b. Preparation of high-potency, bacteria-free suspensions of SR-RSV by a new combination of buffer systems, differential centrifugation, and membrane filtration.

c. Induction of brain tumors in young dogs by injecting the high potency suspensions of SR-RSV.

d. Measurement of antibody responses in tumor-bearing dogs, demonstration of SR-RSV antigen in the induced brain tumors, and recovery of SR-RSV from the tumors in chick embryo fibroblast tissue culture.

e. Measurement of the effect of various drugs on reducing the amount of SR-RSV necessary from brain tumor induction.

f. Morphologic study of the SR-RSV-induced brain tumors and their cells shed into the spinal fluid with histochemical, electron microscopic, light microscopic, and immunoflourescent techniques.

g. Brain scanning of tumor-bearing animals with various isotopes.

Patient Material: None

Major Findings: a. A new method of preparing bacteria-free SR-RSV suspensions containing 10 to 100 times more virus per milliliter than previous suspensions has been developed, with resultant brain tumor induction with only 10^{-1} to 10^{-2} as much suspension as required previously. Small-volume injections of this concentrate allow study of such basic problems as the influence of site of virus injection on resultant tumor type. Also with these potent suspensions, a truly quantitative system is available for the first time to study aspects of crossing genetic barriers with brain-tumor-inducing viruses. Furthermore, the general technique of virus purification has been shown to be applicable to other animal RNA tumor viruses.

b. The complete elimination of bacteria from the virus suspensions for the first time has made SR-RSV-induced brain-tumor-bearing dogs an economically feasible animal model available for study of practical methods of brain tumor diagnosis, which can be directly extrapolated to the human situation.

c. The type of brain tumor induced by SR-RSV, e.g. sarcomas, 7 types of gliomas, and ganglio-gliomas, have been shown to be more dependent on the place in the brain the virus is injected rather than on the type of virus. This phenomenon will have to be taken into account as attempts are made to induce (or "transmit") brain tumors in dogs with cell free extracts from human tumors.

d. Virus-induced brain tumor material from the dogs has been processed for electron-microscopic examination.

Significance to Bio-Medical Research and the Program of the Institute: A method of demonstrating brain tumor induction in animals with cell-free extracts from human brain tumors will be

necessary to prove a viral etiology of human tumors. This study represents an important development in the technique of virus purification, which can in turn be used to process human tumors for attempts at demonstrating their viral etiology, and for the production of vaccine if a viral etiology can be proved.

Development of this relatively low cost brain tumor animal model system will facilitate improvement of methods for diagnosis of human brain tumors.

Proposed Course of Project: The above techniques will be refined further and used to find the most sensitive way to induce brain tumors in dogs by a known RNA tumor virus (SR-RSV), prior to attempts at demonstration of the viral etiology of human brain tumors. Practical diagnostic techniques directly applicable to patients with suspected brain tumors, such as spinal fluid cytology, and brain scanning, will be studied in detail. The electron-microscopic aspects of the virus-induced tumors from the dogs will be examined and compared with human brain tumors.

Honors and Awards:

The principal investigator received the 1969 Resident's Award of the Southern Neurosurgical Society for his paper entitled "SR-RSV induced brain tumors in dogs; neuropathological and immunological observations," which partly covered the studies described herein and partly his investigations prior to coming to the Medical Neurology Branch.

Publications: None

Serial No. NDS(I)-62 MN/NR 925(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Small Sella Turcica

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Thomas Aceto, Jr.
Mary Parker
Alvin Hayles
Colin B. Holman

Other Investigators: None

Cooperating Units: The National Pituitary Agency, Baltimore, Maryland

The Children's Hospital, Buffalo, New York

Washington University, St. Louis, Missouri

The Mayo Clinic, Rochester, Minnesota

Man Years:

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: An anatomo-radiographic study to confirm in a large series of patients the diagnostic significance of the small sella turcica. In addition, we plan to establish whether or not the sella turcica of hypopituitaric dwarfs changes in size after growth hormone treatment.

Methods Employed: Volumetric studies of the sella turcica in many hypopituitaric dwarfs before and after growth hormone treatment.

Major Findings: During last year many more sella turcica measurements of dwarfs have been gathered. A group of these patients have already been checked as far as possible changes of the sella turcica size, after a first treatment course with growth hormone.

Significance to Bio-Medical Research and the Program of the Institute: The rarity of the small sella turcica in the average frequency of this radiographic finding in hypopituitaric dwarfs make this x-ray sign a valuable diagnostic element. The diagnosis of idiopathic hypopituitaric dwarfism in pre-pubertal children is difficult and dependent to a large degree upon indirect chemical estimations of various trophic hormone deficiencies of the pituitary. The finding of a sella volume below the normal range for the patient's age should suggest and should be considered as confirmatory evidence of hypopituitarism in the doubtful cases.

Proposed Course of Project: Evaluation of the gathered data, in particular comparison of the sella turcica measurements before and after treatment with growth hormone.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/NR 1047(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Isotope-Ventriculography and Isotope-Cisternography

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro

Other Investigators: William H. Briner
Ayub K. Ommaya

Cooperating Units: Department of Nuclear Medicine, Diagnostic Radioisotope Section, Clinical Center, NIH

Radiopharmaceutical Service, Pharmacy Department, Clinical Center, NIH

Surgical Neurology Branch, National Institute of Neurological Diseases and Stroke, NIH

Man Years:

Total:	.2
Professional:	.2
Others:	0

Project Description:

Objectives: A gamma emitting isotope injected within the cerebrospinal fluid pathways will permit in subsequent head scans the pictorial outline of the ventricular system (isotope-ventriculography) and of the subarachnoid intracranial spaces (isotope-cisternography). Information about the anatomical status of the cerebrospinal fluid cavities, and, by multiple serial scans, of the normal and abnormal dynamics of the cerebrospinal fluid itself will be obtained.

Methods Employed: We have now expanded the choice of the tracers for isotope-ventriculography and isotope-cisternography. Besides low protein RIHSA and $^{99m}TcO_4^-$, we have introduced high specific activity ^{99m}Tc-HSA. High specific activity tagging of the albumin (up to 12 millicuries per milligram of albumin) has been accomplished by Mr. William H. Briner of our Radiopharmaceutical Service. This new tracer has the following advantages:

1) favorable physical characteristics of ^{99m}Tc; 2) "advantageous" biological behavior of the albumin; 3) lower critical organ (spinal cord and brain) and total body radiation; 4) possibility of effective and efficient use of the gamma scintillation camera. The use of this instrument, recently acquired by the NIH, represents the other important recent improvement in isotope-cisternography. The combination of ^{99m}Tc-HSA and the camera has allowed us to study more efficiently and accurately the cerebrospinal fluid rhinorrheas. We may actually "photograph" the tagged cerebrospinal fluid while it runs out from the subarachnoidal spaces into the nasal cavities. High specific activity RIHSA has not lost its importance as a tracer for isotope-cisternography, in particular in cases where late (24, 48, and 72 hours) records are necessary. In a certain number of patients, both ^{99m}Tc-HSA and RIHSA have been introduced intra-thecally, giving us the opportunity to have early (30 minutes, 1 hour, 2 hours) records (^{99m}Tc-HSA) and late (24, 48, 72 hours) records (RIHSA). The discrimination allowed by pulse height analysis has permitted this double tracer technique. $^{99m}TcO_4^-$ remains the tracer of choice for most of the ventriculographies and the study of the corrective surgical shunts.

Major Findings: The techniques of isotope-ventriculography and, in particular, isotope-cisternography are now well established, routine diagnostic procedures. Hundreds of patients have been studied by these techniques. Traumatic and "spontaneous" cerebro-spinal fluid leaks, leptomeningeal cysts, non-communicating hydrocephalus due to arachnoiditis or subarachnoidal bleedings, the porencephalic cysts and evaluation of neurosurgical shunts remain the main indications of these diagnostic methods. The successful surgery in patients (previously operated on "blindly" and, therefore, often without success) with cerebrospinal fluid leaks and in patients with leptomeningeal cysts and CSF pathways obstructions has been possible because of the diagnostic information obtained with these radioactive scanning procedures.

Recent appraisal of the isotope-cisternographic findings in cases of communicating hydrocephalus of various types (including the so-called occult or normotensive) seems to confirm our original impression that patterns which can be used for a prognosis of the CSF shunting results do indeed exist. In particular, it would appear that early intraventricular penetration of the tracer and persistent and prolonged "pooling" of the radiopharmaceutical in the same ventricles may represent an indication that the patient will benefit, sometimes dramatically, from a CSF shunting procedure. On the other hand, patients in whom cisterno-graphy shows a "temporary" passage and no long stasis of the tracer in the ventricular system do not often improve after

surgical shunting procedures. A cisternographic analysis of the subarachnoidal patterns of distribution is also important in this respect.

Significance to Bio-Medical Research and the Program of the Institute: Legions of authors are studying this remarkable fluid (CSF) which still remains uncomprehended since Cotugno first described it in 1764. In particular, we have now a diagnostic tool to gather information about the "terra incognita" which is represented by the basal and convexity subarachnoidal pathways.

Proposed Course of Project: Further information about the normal and abnormal cerebrospinal fluid cavities, and the normal and pathologic flow of the CSF will be gathered by the techniques of isotope-cisternography and isotope-ventriculography.

Particular attention is being devoted to the study of the various types of hydrocephalus. The techniques of isotope-cisternography and isotope-ventriculography are ideally suited to study the physiopathology of hydrocephalus. From the pragmatic point of view, these isotopic techniques are the best available for deciding the types of hydrocephalus which should be treated with the different types of CSF shunting. A preliminary analysis of our collected clinical material including patients who have been preoperatively (and often postoperatively) subjected to cisternography, makes us feel that we will probably be able to give definite guidelines for certain aspects of the treatment of hydrocephalus.

Honors and Awards: None

Publications:

Di Chiro, G., Ommaya, A. K., Ashburn, W. L., and Briner, W. H.: Isotope cisternography in the diagnosis and follow-up of cerebrospinal fluid rhinorrhea. J. Neurosurg. 28: 522-529, 1968.

Di Chiro, G., Ashburn, W. L., and Briner, W. H.: Technetium Tc 99m serum albumin for cisternography. Arch. Neurol. 19: 218-227, 1968.

Serial No. NDS(I)-65 MN/NR 1195(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Selective Arteriography of Spinal Cord Arteriovenous Aneurysms

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
John L. Doppman
Ayub K. Ommaya

Other Investigators: None

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Surgical Neurology Branch, National Institute of Neurological Diseases and Stroke, NIH

Man Years:

Total:	.2
Professional:	.2
Others:	0

Project Description:

Objectives: Diagnosis of the nature of space-occupying lesions within the spinal canal is often difficult. In most instances with myelography, we are only able to put in evidence a total or partial subarachnoidal block. Myelograms, on the other hand, are seldom helpful in establishing the type of lesion which has caused the block. With recent advances in the field of arteriography--safer radiographic contrast media, better needles, and better catheters, and the revival of interest in subtraction--we feel that a systematic angiographic evaluation of the intraspinal mass lesions is worthwhile. Angiography is of particular importance, of course, in spinal cord vascular malformations. Significant information might, however, be obtained in other types of lesions, especially spinal cord tumors. It is conceivable that tumoral pathologic vessels, and displacement of the large spinal cord arteries (in particular, the arteria radicularis magna--Adamkiewicz) may be demonstrated in cases of spinal cord tumors in analogy to similar angiographic findings in brain tumors.

Methods Employed: Selective arteriograms with the use of modern catheter techniques are carried out in patients in whom intraspinal space-occupying lesions are suspected. The subtraction technique is used to better visualize the injected vessels.

Major Findings: Selective arteriography of the spinal cord is now established as the diagnostic procedure of choice in cases of arteriovenous malformations of the spinal cord. Angiographic demonstration of the arteries feeding these malformations has been possible in over twenty cases. The diagnostic applications of selective arteriography of the spinal cord vessels has been extended to other pathological conditions. Thus, displacement of the arteria radiculo-medullaris magna (Adamkiewicz) and the anterior spinal artery has been observed in six patients with space-occupying lesions (tumors and others) in and around the spinal cord.

Spinal cord arteriography offers in cases of one particular type of tumor--the cord hemangioblastoma--a radiographic picture which has not been found in any other cord lesion. This picture consists of a discrete, roundish area of "blush or stain". No individual separated vessels are recognizable within this "blush". Because of this particular appearance, the hemangioblastomas of the spinal cord may be differentiated from the arteriovenous malformations. Although, in fact, the arteriovenous aneurysms may present angiographically as a congeries of vessels reminiscent of a "ball of yarn", these lesions have never shown, in our experience, the diffuse stain which has been demonstrated in the hemangioblastoma. The arteriovenous malformations will always reveal, at a close scrutiny, the different separated vessels which are the components of the lesion.

The angiographic differential diagnosis between hemangioblastomas and arteriovenous malformations of the spinal cord has pragmatic importance. These two types of lesions, in fact, are treated surgically with a very different approach: Ablation for the hemangioblastomas, and ligation of the feeders for the arteriovenous malformations. The preoperative differentiation acquires, therefore, a significant practical value.

Significance to Bio-Medical Research and the Program of the Institute: Selective arteriography of the spinal cord is increasing our understanding of the large group of conditions in which vascular lesions of the cord represent the basic pathologic element.

Proposed Course of Project: Further technical improvement of the selective arteriography of the spinal cord technique. We are particularly interested in radiographic magnification

using the new fractional focal spots x-ray tubes. The resolution of the small vessels should be improved with these magnification techniques.

We are planning to increase our clinical angiographic experience.

Honors and Awards: None

Publications: Di Chiro, G. and Doppman, J. L.: Differential angiographic features of hemangioblastomas and arteriovenous malformations of the spinal cord. Radiology (In press).

Di Chiro, G. and Doppman, J. L.: Radiology of spinal cord arteriovenous malformations. Progr. Neurol. Surg. (In press).

Serial No. NDS(I)-66 MN/NR 1283(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Empty Sella Turcica

Previous Serial Number: Same

Principal Investigator: Giovanni Di Chiro

Other Investigators: None

Cooperating Units: Diagnostic Radiology Department, Clinical Center, NIH

Surgical Neurology Branch, National Institute of Neurological Diseases and Stroke, NIH

Endocrinology Branch, National Cancer Institute, NIH

Pathologic Anatomy Branch, National Cancer Institute, NIH

Man Years:

Total:	.1
Professional:	.1
Others:	0

Project Description:

Objectives: During previous anatomic work on the sella turcica, "empty" sellae, i.e. instances in which the pituitary may so far fail to fill the sella that the anatomic specimen appears empty, were observed in several cases. The empty sella is usually larger than normal: In fact, in a series of 66 postmortem anatomic observations of the sella turcica and its content, the largest sella was an "empty" one. A selective hydrocephalus of the intrasellar subarachnoid space is considered to be the cause of the large empty sella.

The existence of a large empty sella has pragmatic importance. A large empty sella may mimic an intrasellar tumor and a differential diagnosis has to be made. In addition, the abnormal dilatation of the intrasellar subarachnoid space may cause, probably with a pressure mechanism, a hypopituitaric syndrome

(as in several of our cases) or the development of an intrasellar tension cyst. These cysts may have eroding ability resulting in a break of the sellar floor and an ensuing cerebrospinal fluid rhinorrhea. The empty sella acquires, therefore, significance as a specific pathologic entity. Finally, the knowledge of the existence of a large intrasellar subarachnoid space is important if therapy of the pituitary gland (whether trans-sphenoidal hypophysectomy, trans-sphenoidal ^{90}Y interstitial therapy, or external irradiation) is contemplated.

Methods Employed: Suitable views (hanging-head lateral with autotomography or zonography) are taken at the end of most pneumoencephalographies. Prior to taking these special views the patient is kept in the hanging-head position for at least five minutes, the intent being to fill with air the intrasellar subarachnoid space if present. Particular attention is paid to patients with large sellas and with "dyspituitarism". Occasionally surgical or autopsy follow-up is obtained.

Major Findings: Moderately large to large or very large intrasellar subarachnoid space (cisterna intrasellaris) has been observed in an unexpectedly high number of patients with normal, borderline big, or big sellas. The intrasellar subarachnoid space is located in the anterior part of the sella, with the pituitary gland occupying the infero-posterior part of the sellar cavity. In certain instances the compressed pituitary gland appears to be reduced to a shell-like structure filling only the postero-inferior section of the sella.

Significance to Bio-Medical Research and the Program of the Institute: The knowledge of the large empty sella is important for: a) the differential diagnosis of intrasellar tumor; b) the explanation of certain hypopituitaric syndromes and certain types of spontaneous cerebrospinal fluid rhinorrhea; and c) to avoid mistakes when contemplating sphenoidal (surgical and ^{90}Y) or external radiation pituitary treatment.

Proposed Course of Project: The gathered material needs to be tabulated and evaluated so that the precise meaning of the empty sella turcica in normal and pathological conditions can be established.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 MN/NR 1413(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Radioactive Scanning Tomography of the Brain

Previous Serial Number: Same

Principal Investigators: Giovanni Di Chiro
Floro Miraldi

Other Investigators: None

Cooperating Units: Department of Nuclear Engineering
Case Western Institute of Technology
Cleveland, Ohio

Man Years:

Total:	.2
Professional:	.2
Others:	0

Project Description:

Objectives: Radioisotopic brain scanning is fast becoming one of the essential diagnostic methods to explore the brain. Its reliability in detection and localization of intracranial space-occupying lesions is high (about 80%) but still somewhat lower than that of other neuroradiologic procedures (cerebral angiography, pneumoencephalography). To improve the accuracy of brain scanning, we may essentially follow two research avenues: 1) better tracers; 2) improvement of the equipment. The improvement of the equipment is aimed at increasing the resolution without a substantial loss of sensitivity. This double goal will undoubtedly be reached if a method of layer body section (tomography) associated with scanning can be developed.

Methods Employed: A radioactive brain scan (obtained with a rectilinear scanner) or a scintiphoto (obtained with a fixed device such as a gamma scintillation camera) represents a complex record of signals (photons) originating from the endocranial, cranial and facial tissues. All these signals superimpose in a pattern in which it is often difficult to discriminate between the various anatomical elements. An improved analysis of the scintillographic record of the head would be attained by tomography.

We have been theoretically analyzing the various methods tried by other investigators to solve the problem of scanning tomography. These methods fall into four general catagories: 1) focusing collimator systems; 2) systems using collimators inclined to the plane of interest; 3) scanning with collimators in the plane of interest; 4) time-of-flight positron systems.

We have performed a mathematic appraisal of the three fundamentals parameters: Sensitivity, resolution, and efficiency.

We have proposed a new approach: The strong focusing multi-layered Tomoscanner. Judging from theoretical considerations, this new system holds promises to be, in many ways, advantageous compared to the ones already tested by other investigators.

Major Findings: The response of the already available tomographic scanning systems was calculated, and the resolution determined for the variations of the following parameters: Collimator focal length, collimator thickness, channel exit and entrance radii, and channel or collimator inclination with respect to the body normal. These data indicated the need for new concepts which led to our design of a new device, the Tomoscanner.

The heart of the Tomoscanner is the collimator system which consists of a series of tapered slits angled to the plane of interest with a scintillation crystal at the slit exit. Each plane of interest is examined by a set of slits with each slit tilted in opposite directions from the normal to the plane. The collimator movement is a linear sweep, rotation through an angular increment, and repeat of the motion. Readout consists of film integration of a line sweep on an oscilloscope corresponding to the collimator motion. Construction is simple, and the design incorporates the capacity to examine several layers simultaneously.

Significance to Bio-Medical Research and the Program of the Institute: A successful and simple method of scanning tomography would revolutionize the field of neuroradiology, and parenthetically other fields of radiology. Brain scanning and brain scintiphotography are in fact essentially innocuous (no mortality or morbidity), causing no discomfort to the patient, "less radiation giving" (if proper isotopes are used), and cheaper than other neuroradiologic procedures. Brain scanning and brain scintiphotography are also simple, quick procedures and may in fact, in most instances, be carried out on an ambulatory basis without admitting the patient to the hospital. Last but not least, interpreting a brain scan or a scintiphoto is much simpler and straight-forward than reading a cerebral angiogram or a

pneumoencephalogram. If, therefore, the resolution of brain scanning can be significantly improved by tomography, a complete change-over of the neuroradiology departments will occur. Brain scanning and brain scintiphotography would acquire further diagnostic importance for the detection of intracranial tumors, bleedings, and infarctions following strokes. Some of these lesions--especially the ones of the infiltrating type--which today escape detection by any of our diagnostic neuroradiologic methods, would then probably be "picked up". An increase, in absolute terms, of the detecting accuracy of the neuroradiologic methods taken as a "whole" would then occur.

Proposed Course of Project: We intend to carry out experiments to confirm the validity of the concepts upon which the design of the Tomoscanner is based. These experiments will consist of: 1) Measurement of resolution and sensitivity as a function of depth of the layer of interest for variations in collimator thickness, angulation, taper and width. 2) Appraisal of image presentation with variations in electronic data handling and signal processing.

If the experimental results match and sustain our hypotheses, we will then perhaps proceed with building a prototype Tomoscanner.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 MN/NR 1654(c)
1. Medical Neurology Branch
2. Neuroradiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental Spinal Cord Angiography

Previous Serial Number: None

Principal Investigators: Giovanni Di Chiro
Larry C. Fried
John L. Doppman

Other Investigators: None

Cooperating Units: Diagnostic Radiology Department, National Institute of Neurological Diseases and Stroke, NIH

Surgical Neurology Branch, National Institute of Neurological Diseases and Stroke, NIH

Man Years:

Total:	.4
Professional:	.4
Others:	0

Project Description:

Objectives: The clinical value of the NIH developed technique of selective arteriography in the management of arteriovenous malformations and tumors (in particular hemangioblastomas) of the spinal cord is now well established. It is obvious, however, that two conditions must be met if this method is to become a more advantageously and frequently used diagnostic procedure. The technique needs to be simplified, and its clinical indications must be expanded to include the common and important obstructive vascular disease. To attain the two above-mentioned goals, we have started to work in the area of experimental spinal cord angiography.

Methods Employed: Experimental spinal cord angiography has been carried out in various animals: Primates--monkeys and chimps,--dogs, cats, rats. Our largest experience, however, has been gathered with the rhesus monkey (macaca mulatta): Over 80 such

primates have been used for our work. The remarkable similarity of the anatomical arrangement of the cord arterial supply in the macaque and man makes this species of monkey particularly suitable for this type of research. We have been centering our attention on the thoracolumbar cord, although few experiments have been carried out to evaluate angiographically the cervico-thoracic cord.

The angiographic studies were performed by aortic catheterization. Serialograms were taken in both anteroposterior and lateral projections, and in most instances a direct radiographic magnification technique was used (tube with 0.3 focal spot). Three basic aortographic techniques were used: 1) Conventional, 2) Pressor amines aortography, where the angiographic studies followed the aortic injection of various pressor amines, 200 mcg. of levarterenol base being most frequently used, and 3) External abdominal compression aortography where the angiographic study was carried out after establishing marked (200 to 300 mm of Hg.) external abdominal compression by means of an inflatable balloon.

The angiographic studies have been followed in a large group of animals by surgical exposure and selective ligation of the radiographically visualized vessels. Surgical occlusion of the arteries of Adamkiewicz and of the anterior spinal artery immediately above and immediately below the origin of the Adamkiewicz has been accomplished in many monkeys. Clinical, radiographic and postmortem follow-up of the operated monkeys have been obtained. The anatomo-radiographic observations made at angiography, and the experimental lesions caused by the vessels ligation at surgery, have been matched and controlled with the clinical, gross pathological, microangiographic, and fluorescent substances perfusion techniques. Blood flow "currents" within the large spinal cord arteries have been evaluated by visual and cinematographic controls of the surgically exposed cord after perfusion with vital dyes (coomassie blue, Evans blue).

Major Findings: The spinal cord vessels of the rhesus monkey are only occasionally demonstrated by conventional aortography.

Pressor amine aortography gives a consistent, clear, and detailed radiographic visualization of the spinal cord arteries in the monkey. Also by external abdominal compression aortography, a good demonstration of the spinal cord vessels in the monkey is generally obtained.

Ligation of the arteria radiculomedullaris magna in the monkey does not produce any significant neurological deficit.

Ligation of the anterior spinal artery above the arteria radiculomedullaris magna also fails to cause serious neurological damage. Ligation of the anterior spinal artery below the arteria radiculomedullaris magna leaves the monkey paraparetic or completely paraplegic.

Microangiography is a very valuable technique to confirm and to better understand the anatomoradiographic findings of in vita spinal cord angiography. Microangiography is also of crucial importance to study the various patterns of collateral circulation after experimental occlusion of the different spinal arteries.

Studies with fluorescent substances hold some promise to be useful in the appraisal of the extent of the areas of the cord involved in the various types of experimental vascular damage.

We have demonstrated by vital dyes angiography that "ascending and descending currents" of the blood flow do indeed exist in the large spinal cord arteries of the rhesus monkey.

Significance to Bio-Medical Research and the Program of the Institute: Our results with experimental spinal cord angiography offer the basis for attempting to develop similar spinal cord angiographic techniques in man. These techniques would, in turn, allow a more careful clinico-radiographic analysis of many pathological conditions of the spinal cord. The demonstration that the ligation of the arteria radiculomedullaris magna leaves the macaque intact has important implications for the surgical treatment of various intraspinal lesions affecting the territory near this important artery.

Proposed Course of Project: We plan to gather further experience with the techniques of pressor amine and compression aortographies. In particular, we are going to try new types of pressor amines, different dosages, and "cocktails" of the various drugs. We also intend to test the usefulness of some "protective" substances (glucose, mannitol dextran, procaine). Various modifications of the compression technique will be tried. When we are convinced of the reasonable safety of these new experimental techniques, we will start to apply our acquired knowledge to human clinical material.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 MN/AP 926(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electromechanical Coupling in Muscle and Drug Activity

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B. S.

Cooperating Units: None

Man Years:
Total: 1.95
Professional: 1.95
Other: 0

Project Description:

Objectives: The sequential pattern of physiological events which develop in skeletal muscle starting with excitation and ending in relaxation has not been adequately defined. The molecular interactions that link excitation to contraction in the physiological state are largely unknown. Not much knowledge has accumulated about the molecular changes involved in the uncoupling of contraction from excitation which permits relaxation. The object of the present study was to establish an ionic basis for the initiation, maintenance, and subsequent loss of tension in slow-type skeletal muscle and to relate the ionic events to electrical, mechanical, and metabolic activity.

Methods Employed: The coupling and uncoupling processes between the tension-relaxation cycle and the related ionic events are studied in slow-type skeletal muscle fibers. This kind of skeletal muscle occurs in several species including human beings, monkeys, cats, rabbits, and frogs. Since classical electrophysicological and mechanical methods have by themselves failed to explain fully the coupling processes, ion substitution methods have been developed and used. A sucrose gap technique using pairs of isolated muscles and continuously flowing ionic solutions has been developed and used operation-

ally to determine electrical membrane changes. A di-monovalent di-buffer amine has been found which sufficiently maintains osmalarity at a physiological pH and serves precisely as a monovalent, nonpenetrant, and electrical-charge substitute for both Na^+ and Cl^-.

Major Findings: This section of the 1967-68 report stated the experimentally derived evidence that in slow muscle Na^+ current is a sequential step between excitation and tension development. During the past year our research data have defined the coupling events between contraction and relaxation in slow skeletal muscle. The new findings are that relaxation depends on the outward active transport of Na^+ mediated by the Na^+-K^+ activated adenosine triphasphatase. The evidence is as follows: (1) Concentrations of ouabain which inhibit Na^+-K^+ ATPase induce a delayed contraction in slow muscle. The delayed contraction does not occur in ouabain solutions if the Na^+ has been replaced by monovalent nonpenetrant cations. (2) After slow muscle has been contracted by Ca^{++} depletion in Na^+ solutions the return of Ca^{++} promptly relaxes the slow muscle. Relaxation is prevented by ouabain. (3) Slow muscle made to contract by Ca^{++} depletion relaxes rapidly when the Na^+ of the Ca^{++}-free solution is replaced by a monovalent nonpenetrant cation. Ouabain prevents this rapid relaxation. (4) The experiments indicate that ouabain selectively inhibits the slow muscle Na^+-K^+ activated transport ATPase but does not affect actomyosin Mg^{++}-activated ATPase or the Ca^{++} dependent ATPase necessary for relaxation. (5) When slow muscle is kept in an isotonic solution of Li^{++} ions which are not actively transported outward by Na^+ -K^+-ATPase a delayed contraction develops. The return of Na^+ relaxes the slow muscle. (6) Slow muscle kept in isotonic Li^{++} by Na^+ induces relaxation. (7) The slow muscle responses described in (1) through (6) above occur in denervated muscle and thus are myogenic in origin. (8) Slow muscle fails to relax completely when deprived of external K^+. Failure of relaxation is increased by prolonged K^+ depletion which leads to internal Na^+ accumulation. Na^+-K^+-ATPase is oriented across membranes and is stimulated by external K^+ and internal Na^+. Decreased external K^+ reduces Na^+ transport in muscle. (9) The rate limiting process in contracture of slow muscle in response to Ca^{++} depletion is Ca^{++} diffusion which has a low temperature coefficient of below 2.0. Relaxation of slow muscle has a high Q_{10} of 4.0 which is inconsistent with ion diffusion and consistent with active transport. (10) Conditions which produce relaxation in slow muscle do not change the resting membrane potential in fast muscle. These research findings constitute the first description of a relationship between contractile function and electrogenic ion transport in muscle tissue.

Slow muscles contract when the ionic strength of their external fluid approaches zero and relax in the absence of Ca^{++} when ionic strength approaches one. This type relaxation depends on ionic strength and not penetrability of either external anions or cations. A further definition of the basic contraction-relaxation events is necessary.

Some ionic events of the excitation-contraction-relaxation cycle in mammalian smooth muscle have been defined.

Significance to Bio-Medical Research and the Program of the Institute: The research of this project continues to furnish new data of a fundamental nature related to muscle function and malfunction. For practical reasons this project utilizes slow muscle from lower forms as an approximation to the slow (tonic) muscle found in human beings and other mammalian species. This project has the significance of the discovery of new fundamental knowledge in an area where the pathologic physiology of the disease entities are poorly understood or treatable.

Proposed Course of Project: The work of this project should continue as long as it yields new knowledge about a tissue system in which disease processes occur that are not fully understood.

Honors and Awards: None

Publications: Irwin, Richard L., and Oliver, Katharine L.: Sodium induced tension in slow skeletal muscle deprived of Ca^{++}. Am. J. Physiol. 217, No.1. In Press.

Serial No. NDS(I)-63 MN/AP 1049(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Mechanical Properties of Muscle in Relation to Drug Action

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.3
Professional: 0.3
Other: 0

Project Description:

Objectives: To a sufficient degree, one of the original aims, to characterize the contractile properties of mammalian muscle, has been realized. It was planned to apply these results in the evaluation of certain drugs known to affect muscular contraction.

Specifically, inquiries are continuing into the elastic properties of active skeletal muscle. An investigation has been initiated to determine the influence of drugs, known to affect the neuromuscular apparatus, on the contractile properties of skeletal muscle.

Methods Employed: The emphasis of these studies on mechanical responses of muscle has indicated a methodology based on mechanical recording techniques. Tension and displacement transduction systems have been developed with considerable attention toward their properties which in turn increased the fidelity of the muscle measurements and subsequent interpretation.

Major Findings: The currently accepted concept of a passive elastic element joined in series with an active contractile component was not supported by data derived in this laboratory from in situ mammalian muscle.

Significance to Bio-Medical Research and the program of the Institute: The mechanical characteristics and contractile properties of active muscle serve as the standard for evaluation of the effects of disease, environment, drugs, etc., on contractile responses. This project is designed to expand, clarify and modify, when indicated, the present conceptual models in terms of these characteristics.

Proposed Course of Project: An extension of the present studies to other species and types of contractile systems is planned.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 MN/AP 1050(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Functional Activity and Mechanical Properties of Striated Muscle

Previous Serial Number: Same

Principal Investigator: Jay B. Wells, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.7
Professional: 0.7
Other: 0

Project Description:

Objectives: Investigations were conducted on the relationship between muscle contractile responses and mechanical or neural environment and on adaptive changes in these responses which migh occur with changed environmental conditions. Specifically, the three areas of interest which were investigated included: (1) Classification or indexing individual mammalian skeletal muscles based on contractile speed. (2) Investigation of the effect on contractile properties of modification of the physical environment. (3) Evaluation of the effect on mechanical responses of modification of the neural input to a postural muscle.

Methods Employed: Since the three inquires were concerned with the mechanical responses of normal and modified muscle, evaluation of muscle contractile properties was based on measurements from the active contractile elements only. Therefore, dynamic contractile characteristics were derived by applying data from isotonic contractions to a widely used mathematical model of muscle mechanics. The resulting characteristic constants, which are related to either the amount or rate of contractile energy output, formed the basis for the comparison between individual muscles.

Major Findings: Values for b, the characteristic constant related to the rate of contractile energy output and V_o, the intrinsic shortening velocity, were derived from various limb muscles in 38 animals. Muscles composed mainly of fast fibers such as the digital extensors or fast flexors showed large values for these constants while corresponding values from slower soleus muscles were low. A correlation was found between the values for the two characteristic rate constants and corresponding isometric tension rates (currently the most commonly used estimator of muscle speed). Data for the correlation were obtained from fast and slow muscles of rats, guinea pigs, cats, and kittens. A similar correlation was obtained between values of the rate constants and isometric twitch time (another widely used estimator of muscle speed). The constant b rather than V_o was indicated to be the better possible index of muscle speed. Such an index, if established, should find application in both clinical and fundamental muscle studies. Mathematical derivation of the relationship between b and corresponding isometric tension rates from these studies will permit calculation of the index from easily measured isometric contractions. In addition to extending the observations to other muscles and species, correlations between derived b values and muscles with known fiber type composition are planned.

The following summary of major findings concludes present investigations on the influence of mechanical (environmental) factors on the contractile responses of voluntary muscle. The mechanical environment of rat limb muscles was modified to preclude active shortening, a basic response of all contractile tissue, by joint fixation. Thus, functional disuse was imposed on the muscles without impairment of innervation or capacity for tension development. Wasting assessed by weight loss, was produced in the immobilized muscles. Dynamic constants derived from force-velocity measurements taken 3 - 6 weeks after muscle immobilization were entirely similar to those observed from sham treated muscles. Thus, muscle deprived of shortening but capable of tension development, maintained normal contractile characteristics despite significant wasting and decreased tension output.

The effect of innervation on the contractile response of voluntary muscle was investigated by reinnervation of soleus muscles with the nerve which normally innervated medial gastrocnemius muscle. The cut ends of the motor nerve to soleus were self-reunited and served as controls. The characteristic contractile constants, derived from isotonic responses and measured 10 - 16 weeks after nerve reunions, observed from self-rennervated muscles did not differ from those observed in normal

muscles. However, the contractile constants b (energy rate) and a (amount of energy output) derived from cross-reinnervated muscles were higher than control values. These values approximated corresponding values observed in rapidly contracting muscles. The capacity for tension development was normal and equal in the compared groups. Because these data are derived only from the active contractile element, it is concluded that motor innervation influences and modifies the contractile apparatus of skeletal muscle. Completion of data analysis and preparation of the results for publication will conclude anticipated work in this area.

Significance to Bio-Medical Research and the Program of the Institute: The clinical significance of histochemical "typing" of muscle fibers into distinct groups has indicated the need for such investigation on the mechanical properties of these fiber types. Also, experiments designed to evaluate activity deprivation effects on muscle function have become significant, in addition to their clinical implications, to space and undersea exploration programs.

Proposed Course of Project: An expansion of the studies relating contractile speed to a derived rate constant is planned for muscles with "mixed" fiber type. An extension of the investigations on the elastic properties of active muscle into a variety of muscle preparations is proposed.

Honors and Awards: None

Publications: Wells, Jay B., Functional integrity of rat muscle after isometric immobilization. J. Exper. Neurol. In Press.

Serial No. NDS(I)-67 MN/AP 1416(c)
1. Medical Neurology Branch
2. Applied Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: A Pharmacological and Toxicological Study of Neurotoxic Venoms

Previous Serial Number: Same

Principal Investigator: Richard L. Irwin, Ph.D.

Other Investigators: Katharine L. Oliver, B. S.
Ahmed Hassan Mohamed, Ph.D.

Cooperating Units: Department of Physiology, Ein Shams University, Cairo, Egypt

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives: To obtain and investigate heretofore unavailable venomous substances. Attempts are being made to first determine the primary point of biologic activity and then to find out the tissue and cellular mechanisms of neurotoxic activity.

Methods Employed: The above objectives demand the use of a wide variety of whole animal, isolated tissue, and cellular techniques involving toxicological, pharmacological, and biochemical procedures. One or two well-known venoms in each genus are investigated in order to establish a standard. A comparison of the activity of the venoms from the more rare species aids the discovery of new properties of the various venoms with a minimum of search.

Major Findings: Since this project has been inactive during most of the year there are no new research findings.

Significance to Bio-Medical Research and the Program of the Institute: Past investigations of venoms have been pre-

eminently successful in various aspects of biology.

The collection of venoms from poisonous species has made possible the scientific development of antivenins for the treatment of envenomation by poisonous animals. The research here is collaborative with and supportive of a program that is now designed to collect venoms from the entire African Continent (except for the Union of South Africa) and to develop polyvalent antivenom for therapeutic use.

Because of the accelerating toxicity to which modern man is exposed, the study of toxic and poisonous substances becomes of increasing importance. Only a few relatively common venoms have been investigated. Over 600 different species of poisonous snakes are believed to exist in the world. What wealth of biological tools are undiscovered in the venom of the lesser known species can only be surmized from the success of past research. The relationships among snake venom neurotoxins, enzymes, antivenins, toxicities, and therapeutics are almost completely unknown. The project, therefore, has excellent possibilities of increasing progress in medical research.

Proposed Course of Project: The collaborative program which makes this research possible has not been receiving PL-480 support during the past year. Funding has recently been approved and the entire project can now be evaluated as to its future course.

Honors and Awards: None

Publications: None

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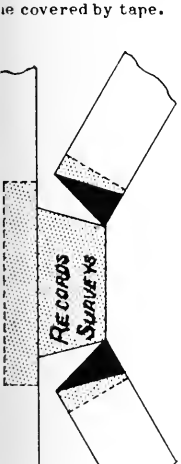

TABBED SEPARATOR SHEET
Form HEW-69
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SURGICAL NEUROLOGY BR.
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NEUROPHYSIOLOG
LABORATORY OF
NEUROCHEMISTRY

ANNUAL REPORT
July 1, 1968 through June 30, 1969
Branch of Surgical Neurology, IR
National Institute of Neurological Diseases and Stroke

Maitland Baldwin, M. D., Chief

During the period of this report, investigations have been conducted under the following categorical titles: cerebral ischemia, cerebral trauma, interhemispheral relationships, language and memory, developmental defects, experimental hydrocephalus, vascular pathology of the spinal cord, epilepsy, cerebral edema, and tumor. Twenty-three reports were prepared for publication in appropriate journals.

During the period 16 April 1968 through 15 April 1969, 203 persons participated in clinical investigations as inpatients, while 566 were examined as outpatients in a total of 777 visits. There were 132 operative procedures, and 66 patients underwent physiological monitoring in the neurosurgical suite.

In the course of these investigations, there was active collaboration with the following organizations: Branch of Electroencephalography, NINDS; Laboratory of Neurochemistry, NINDS; Section of Psychological Research, St. Elizabeth's Hospital; Section of Neuroradiology, Medical Neurology Branch, NINDS; Biometrics Branch, NINDS; Departments of Diagnostic X-ray and Clinical Pathology, Clinical Center; Computer Facilities, DCRT, NIH; Biomedical Engineering and Instrumentation Branch, DRS, NIH; Naval Ship Research and Development Center, Department of the Navy.

Cerebral Ischemia

The development of a laboratory model for cerebral ischemia and cross circulation in the primate continued. Surgical feasibility has been demonstrated and viable preparations with brain temperatures, arterial-jugular oxygen-CO_2 differences, EEG, corneal reflex, pupillary reflex, and movements of lips and jaws have been maintained for periods of 4 to 6 hours after transfer. In the dog, exsanguination ischemia has been followed by survival in 80% of subjects, consequent to application of head surface hypothermia and intrafemoral perfusion 10 minutes after clinical death.

Cerebral Trauma

The natural frequency of brain rotation, injurious effects of whiplash injury, inclusive short duration rotation velocity thresholds for head impact, as well as whiplash injury, and the energy thresholds for concussion have been studied in the monkey. Data for whiplash injury and for the energy thresholds for cerebral concussion bear out last year's prediction. Thus, the information derived from the squirrel monkey and the chimpanzee with relevant average brain weights of 30 grams and 400 grams respectively appears in predicted levels.

Last year's study of occult hydrocephalus following trauma continues and now is based on an aggregate of 30 patients. Data derived suggest that

only such individuals showing stasis of isotope in cisternography will improve after shunting.

Interhemispheral Relationships

Studies of normal subjects and patients (individuals with definable non-neoplastic lesions localized to one or the other hemisphere) have been made using standard scalp electrode placements, the Grass integrator, and verbal and non-verbal skilled tasks. When a normal person, who is right handed and suspected of having left dominance for verbal speech, begins a verbal task, there seems to be some early rise in right hemispheral power levels which is then followed by a relative increase in left hemispheral power levels which predominates during the course of the verbal task. Generally speaking, the reverse is true when a non-verbal task is presented to a similarly right-handed person. In patients whose Wada test provides some more effective lateralization in verbal speech to one or the other hemisphere, there is correlation between lateralization of verbal speech, verbal skilled task, and left hemispheral power level.

Visual discrimination of verbal and non-verbal stimuli is accompanied by dissimilar evoked potentials from left and right occipital lobes. Words evoke greater differences in average responses from left hemisphere, whereas the right hemisphere reflects greater differences during perception of word and design stimuli. In each instance, the inter-hemispheric differences are manifested in the shape of latency of evoked potentials. In addition, maze performance by patients with multiple contact electrodes situated in the thalamus or cingulum yield differential evoked responses which correspond to correct or incorrect performance. Evoked responses recorded from right pulvinar show distinct wave form, most notably for incorrect selections. No evoked pattern was evident from other sites. In one patient with bilateral cingulum electrodes, evoked response was elicited from both cingula, again in association with performance.

In the laboratory, the studies of electroencephalographic differences after mesial cerebral incision have continued as of this report. A surgical preparation in the macaque has been developed in which mesial cerebral, cerebellar, and brain stem incision has been extended throughout the length of the spinal cord.

Language and Memory

After left thalamotomy, male patients made more errors in choosing the most popular word association in the standard word association test, whereas other surgical patients did not.

By using Horst's new method, common and general factor scores were calculated for 42 patients in order to clarify relationships between test scores and extent of neuropathology. Factor loadings were determined from test intercorrelations obtained from 146 intelligent patients tested with 3 standard tests. The extent of surgery or neuropathology on the left was related to scores on a verbal factor, and on the right to scores on a closure factor.

For 5 patients, bilateral cingulum electrode stimulation of the left cingulum was associated with marked impairment in memory for verbal memoranda. No comparable changes in memory accuracy followed right cingulum stimulation, whereas stimulation in the left thalamus, specifically the pulvinar, produced anomia. Cingulum stimulation failed to modify naming behavior. One patient with an electrode directed toward the right amygdala showed no changes in the name-recall performance during stimulation. In addition, patients with left temporal involvement by an epileptogenic process showed learning and memory deficits on verbal tasks, while their performance on non-verbal tasks was unaffected. The converse appeared to be true for patients with right temporal lobe epilepsy. In contrast to the two temporal groups, children with subcortical or centrencephalic epilepsy were without significant memory deficit. Instead, they earned lower scores on a test of sustained attention. The results are similar to those obtained in studies of adults with comparable cerebral dysfunction.

Anatomical studies of cases with loss of recent memory and spatial disorientation based on the material of 5 hemispheres from 3 individuals with a specific infarction of the posterior cerebral artery and destruction of the posterior portion of the hippocampus have been started. Lesions of hippocampal gyrus, lingual gyrus, and small extensions to inferolateral pulvinar and nucleus ventralis caudalis were present, although inconstant. In all material, the fornix showed an extensive degeneration and demyelinization throughout its length. In one particularly well-stained case, it was possible to follow the demyelinated, heavily gliosed bundles from the descending branch of the fornix upward and backward under the stratum zonale of the thalamus anteromedial to the anterior nuclei and also in the myelinated band between the anterior nuclei and the subjacent thalamus. The coincidence of spatial disorientation and recent memory deficits in this relatively extensive material has once more reemphasized the importance of the hippocampus and its central connections in the recording of newly acquired material.

Developmental Defects

A method for determination of molecular weights and characteristic elution diagrams of individual acid mucopolysaccharides in normal controls and affected patients was developed. Elution volume and the logarithm of molecular weight are related in a linear fashion.

Eight such patients had AMPS inclusions in a proportion of the lymphocytes. While such cells do not differ in biological behavior from those without inclusions and the ratio varied considerably in different patients, it was generally higher than in those who were heparitin excretors. On the basis of composition of the acid mucopolysaccharide secreted in urine, the mode of inheritance of the trait can now be classified.

Chondroitin sulfate B and heparitin sulfate have been identified in the cerebrospinal fluid of these patients, with only small traces of chondroitin sulfate A and C. It appears that the amino acid composition of the peptide bonded to AMPS moiety is strikingly similar in all three variants of Hunter-Hurler's syndrome. Predominant amino acids include serine, glycine, and aspartic acid, while the amounts of these three amino acids relative to

hexosamine in AMPS of these patients are also very similar, thus indicating that chondroitin sulfate B and heparitin sulfate are associated with similar peptides.

Experimental Hydrocephalus

Obstructive hydrocephalus was produced in 256 rhesus (Macaca mulatta) monkeys by obstruction of the fourth ventricle and caudal aqueduct with an inflatable balloon. Significant and relevant changes were found in all animals 60 minutes after obstruction. After 3 hours, these were well developed. Thereafter, ventricular size continued to increase, although at a slower rate. It appears that the rate of enlargement is precipitous between 3 to 6 hours, but between 24 to 48 hours, enlargement occurs at a considerably reduced rate. Then it continues very slowly. The early enlargement of the ventricular system was characterized by preservation of the angles and contours, but after 14 hours, the angles became rounded and the specimens derived thereafter showed generalized remodelling of cerebral architecture. Once blunting of angles has occurred, ependymal rupture occurred and intraventricular dye seeped out into the parenchyma.

Effects of Ionizing Radiation

152 female mice were irradiated on consecutive days of pregnancy between the 7th and 18th gestational days. This resulted in 876 irradiated newborns. General exposures to irradiation on the 7th and 8th gestational days led to death and absorption of the majority of the fetuses. However, those which survived were largely normal at birth and only a small proportion had organic-type brain abnormality. Radiation on the 9th gestational day produced an increased number of cerebral abnormalities which included dysraphism, hydrocephalus, microencephaly, and arrhinencephaly.

Epilepsy

Eight children with idiopathic epilepsy were placed on a high fat diet and all of them showed better control of seizures than had been possible on varieties of anticonvulsants.

Thirty patients with intractable epilepsy, otherwise unresponsive to prolonged periods of anticonvulsant medication and outside the reach of conventional surgery for epilepsy, have been subjected to the combination of hypothermia and intravenous dilantin. Eight of these have been followed for periods ranging from 1 to 7 years. Two of these have been seizure-free for 3 and 7 years respectively, and the remaining 6 are reported as having a reduction in seizure frequency greater than 50%. Studies of plasma and CSF levels of dilantin related to normothermia and hypothermia do not show any significant quantitative differences.

Some of the problems raised by the hypothermia-dilantin therapy have been studied in the laboratory. C^{14}-labeled dilantin has been used in some preparations, and in others, C^{14}-labeled curare. The latter was selected and has been successfully used because of its cold-stable characteristics, and because of its dramatic neurological effects if abnormally deposed in

the brain. At this time, it seems likely that abnormal and maximal depositions of curare can be obtained during hypothermia if both extravascular and intravascular cooling are used so as to produce low blood temperatures with a surrounding cold brain. While it is not necessary to fall below an absolute temperature of 25° C, a rapid linear fall to this absolute level in both cerebral parenchyma and blood is critical for maximum deposition of the drug across the barrier. No further information has been derived on the paradoxical finding that the amount of dilantin deposed in hypothermia does not, in fact, exceed that deposed in normothermia. Nonetheless, the dilantin deposed in a hypothermic condition seems more effective against the experimental epileptogenic lesion.

Vascular Pathology of the Spinal Cord

Employing selective aortographic techniques for radiological demonstration of arterial blood supply of spinal lesions has provided for extradural and often extraspinal ligation of arterial blood supply.

Twenty-seven patients have been studied to date. Once the arterial blood supply is clearly demonstrated through selective catheterization of intercostal or lumbar aortic branches from which the pathological vessels may arise, the present technique provides for exposure and intradural ligation of the abnormal supply.

In the laboratory, retrograde catheterization of femoral arteries provided angiographic outlines for principal spinal vessels so that effects of selective ligation of collateral circulation could be studied.

Technical Developments

The laminar flow prototype designed for use over an operating table or bed has been tested against previously developed microbiological surveillance techniques within the neurosurgical suite. This type of apparatus will be useful in caring for the patient who requires isolation on the ward, or as a developmental step towards simplification of laminar flow systems for neurosurgical operating rooms. It was found that particles being shed by attending personnel working within the stream were largely collected by samplers outside its configuration. The high air flow area provides fewer organisms to the sampler even though as many as 4 people with exposed skin were working within it. There is an exodus of skin particles and concomitant microorganisms immediately peripheral to the airflow system which can provide contamination to adjacent unprotected patients or areas.

Serial No. NDS(I)-54 SN/OC 100(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Epileptogenic Mechanisms in the Brain of Man and Other Primates

Previous Serial Number: Same

Principal Investigator: Maitland Baldwin, M.D.

Other Investigators: J. M. Van Buren, M.D., A. K. Ommaya, F.R.C.S., L. Frost, Ph.D.

Cooperating Units: CC-CP; N-OB; PHS-SEH

Man Years
Total: 3.5
Professional: 2.0
Other: 1.5

Project Description:

Objectives:

a. To study causal mechanisms of epileptic seizures in man and other primates.
b. To study the electrographic characteristics of epileptogenic activity in the brain of man and other primates.
c. To study the approved methods of surgical therapy for these lesions and develop new therapeutic methods.

Methods Employed:

a. Clinical neurological examination.
b. Special radiographic and other contrast examinations.
c. Electrographic, including electrocorticographic, examination.
d. Electrophysiological techniques as indicated.
e. Experimental epileptogenic lesions.
f. Hypothermia.
g. Cerebral drug deposition and scintillation counting of tagged drugs.
h. Histological and chemical examinations as required.

Major Findings: At the time of this report, 30 patients have been subjected to a combination of intravenous dilantin and cranial hypothermia as previously described. Eight of these have been available for longer than one year follow-up of which two have had no further seizures for 7 and 3 years

respectively, while 6 out of the 8 show more than a 50% reduction in prior seizure frequency. Plasma and CSF levels taken before, coincident with, and after the cooling process do not show values significantly greater than those reported in the literature for comparable doses at normal temperature.

Temporal lobectomy and other focal excisions have been undertaken for epilepsy and have provided further data for cortical physiology. It should also be noted that these patients, with temporal lobe epilepsy, provide most of the data for the language and memory studies which have been noted elsewhere.

In the laboratory the enigma of the cold permeability relationship has been pursued, and it seems clear from this year's work that the most effective combination of heat exchange is that of surface and intravascular cooling. Thus, when dogs are subjected to intravascular and cranial surface cooling, there are significantly greater amounts of labeled curare in the brain than when the dogs are subjected to general cooling, specific surface head cooling or intravascular cooling alone. There is no question that at the low temperatures there is greater deposition generally than at normal temperatures. Nonetheless, the quantitative differences between the counts of C^{14}-labeled dilantin at normal temperature and those at low temperatures are not significantly different, and the presumption is that hypothermia and the dilantin have a qualitative rather than a quantitative effect.

Significance to Bio-medical Research and Program of the Institute: This project attempts further understanding of epileptic mechanisms, with particular reference to therapeutic applications, and the exploitation of the surgical treatment of these conditions for cortical physiology and for cerebral physiology and behavioral mechanisms.

Proposed Course of Project: The project on dilantin-hypothermia will be continued, as will the surgical investigations.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-54 SN/OC 101(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Functional Representation in the Temporal Lobe of Man and Higher Primates

Previous Serial Number: SAME

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: J. M. Van Buren, M. D., P. Fedio, Ph. D., H. Lansdell, Ph. D., R. Farrier, M. D., R. Wadeson, M. D., L. Frost, Ph. D., R. Mejia, B. S.

Cooperating Units: M-LP, N-OB, CC-Dir., PHS-SEH, CR-DSA

Man Years

Total	1.8
Professional:	1.0
Other:	0.8

Project Description:

Objectives: Further understanding of a significant functional representation within the temporal lobe and related structures in man and other primates.

Methods Employed:

a. Electrical stimulation and recording from human, chimpanzee, and monkey temporal lobes; directly, after operative exposure; indirectly, by depth electrodes and scalp recordings.

b. Ablation of all or parts of the temporal lobes and limbic systems.

c. Surgical interruptions of connecting pathways.

d. Use of drugs for stimulation and suppression, or other effects, on these systems.

e. Test situations, utilizing machine or automatic programming.

f. Other psychological and physiological examinations.

Major Findings: These have been related to language and memory, interhemispheral function in patients, and interhemispheral function in monkeys and chimpanzees. Normal controls and patients have been subjected to verbal and non-verbal testing during scalp electroencephalography with standard electrode placements. The latter records have been subject to integration using the Grass integrator. Attempts have been made to correlate handedness, speech lateralization (as predicted by Wada test), and relative power levels. When a right-handed person is presented with a verbal task, power levels are at first comparable; then there is a slight elevation of the right hemispheral level as compared to the left, and as the task develops, the left hemispheral level exceeds that of the right. In a left or right temporal lobectomized person, there is not, unexpectedly, a reduction in relative power levels on the side of the lobectomy, and this seems irrelevant to the relative increases which may be noted on verbal and non-verbal testing. Patients with known dysnomia, relevant to non-neoplastic cerebral lesions in the left or dominant hemisphere, have also been subjected to this test array. They do not show the same relative rise in power level as normals when presented with verbal tasks. Normals show a relative rise in non-dominant or right hemispheral power levels when presented exclusively with a non-verbal task. This seems to be the case also in patients who have left dominant lesions characterized by dysnomic errors--that is to say, when presented with a non-verbal task, there is still some relative increase of the right hemispheral power levels.

Finally, retrospective analysis of EEG records taken from right and left temporal lobectomized patients who have undergone operation for temporal epilepsy 10 years ago, are being undertaken using the IBM 360/50 techniques noted above. In addition, some of these patients are being recalled in a comparable set so as to examine them with standard scalp placements and then, using the programmer, compare relative power levels in an attempt to relate these to site of operation, site of dominance, and handedness.

A three-week-old, male chimpanzee has been subjected to recordings before, during, and after mesial cerebral incision. These have been programmed in accordance with the method (described in the last annual report) which utilizes strip charts, digitized by means of a Gerber X-Y scanner or a punch-card output suitable as input for the IBM 360/50. This animal, which is doing well and shows no obvious behavioral impairment or neurological symptomotology, is being progressively followed as he ages. There are obvious asymmetries between right and left, but they are not always biased to one side. His arousal-drowsing pattern seems normal, and there is no evidence for selective sleep patterns lateralized to one or the other hemisphere. However, adequate sleep recordings have not been done.

Similarly, mesial spinal incisions have been undertaken in macaque monkeys which have been subjected to pre- and postoperative testing. In essence, the total procedure, which intends the mesial incision of the major portion of the nervous system, is feasible surgically and produces a viable, active preparation which shows high levels of anesthesia to pin, but does

not act as if he is anesthetic or even paresthetic.

Significance to Bio-medical Research and Program of the Institute: These findings relate to such cerebral functions as language, memory, and sensation.

Proposed Course of Project: We will continue study of the interhemispheral relationships using the program established, as well as psychophysiological testing patterns designed for conditioning of one hemisphere and the testing of both. A model of the mesial neurological incision will be pursued.

Honors and Awards: None

Publications:

Baldwin, M. and Mejia, R.: Effects of unilateral section of the brain stem after mesial cerebral incision. Ann. N. Y. Acad. Sci. (In press)

Serial No. NDS(I) - 55 SN/OC 200(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Involuntary Movements

Previous Serial Number: Same

Principal Investigators: J. M. Van Buren, M. D., Choh-luh Li, M. D., and Paul Fedio, Ph.D.

Other Investigators: R. A. Ratcheson, M. D.

Cooperating Units: None

Man Years

Total:	1.6
Professional:	1.1
Other:	0.5

Project Description:

Objectives: Neurological disease characterized by dyskinesias offers a twofold opportunity for research. The pathological aspects of the disease itself may be studied as well as the pathophysiology of the motor system. The second aspect is the unparalleled opportunities afforded for neurophysiological studies in man by stereotaxic surgery in the treatment of dyskinesias. Studies made directly of the disease itself have not received great emphasis in the present research since they require biochemical and pathological support.

Methods Employed: Surface and depth microelectrode recording of the human brain makes use of specially built high impedance pre-amplifiers, tape recording of signals and eventually averaging and cross correlation of cellular activity and other functions such as muscle activity and brain potential variations from large electrodes. Depth electrode stimulation employs a specially built electrode and a current monitored stimulator. The special apparatus used in quantitative psychological testing is described in Dr. Lansdell's and Dr. Fedio's Project Report Serial No. NDS(I) - 63 SN/CP 1033(c).

Major Findings: Apart from the further collection of material, no correlative work has been done due to the preoccupation of one of the principal investigators (J. M. Van Buren) with the thalamic atlas which is needed to form the anatomical support for all stimulation and recording studies.

Over the past few years the importance of adding an investigator with engineering and mathematical skills to the team has been repeatedly stressed. Specifically, a reasonable attack on the problems of human motor function cannot be undertaken without the aid of such an individual.

The possibility of obtaining useful collaborative help has been investigated, but this is not a useful approach since time is required by such an individual and it is not possible to discover those on the reservation willing to devote sufficient time to the project in collaboration with the Surgical Staff.

Lateralization of subcortical mechanisms for verbal and nonverbal memory functions was studied during intracranial stimulation. Electrical stimulation was delivered through chronic indwelling electrodes directed through a parietal burr hole toward a therapeutic thalamic target in patients with motion disorders. Stimulation sites were plotted within the lateral thalamus and the paracallosal area of the left and right hemispheres. Behavioral observations were monitored on a serial object naming task and a random form discrimination test, each with a short term memory command. Performance trials associated with aphasia or reports of sensation were not included for analysis.

A significant impairment in short term memory for verbal memoranda accompanied stimulation in the left superior posterior thalamus; short term memory on the nonverbal task was seemingly spared during left thalamic stimulation. Conversely, stimulation in the homologous region in the right thalamus elicited nonverbal memory impairment while sparing recall performance on the verbal task. Stimulation in the deep parietal white matter in both hemispheres resulted in memory disruption on the verbal and nonverbal tasks. The findings suggest that an asymmetry in the organization of verbal and nonverbal memory processes appears to exist at the thalamic level.

A chapter, "Technical Principles in Stereotaxic Surgery," is being prepared with Dr. R. A. Ratcheson at the request of Dr. Julian Youmans for inclusion in a textbook for neurosurgeons. It will cover in detail the factors in localization including variations in human diencephalic anatomy, a compilation of the various stereotaxic apparatuses, physiological adjuncts to localization in the brain and methods of lesion production. The bibliographic data has been compiled and the manuscript begun for a 1 June 1969 deadline.

Significance to Bio-medical Research and the Program of the Institute: The use of a human subject who is cooperative and unsedated permits many tests of sensory and psychological function which are impossible even with the time consuming conditioning experiments applied in lower animals. The use of the human subject is therefore not a duplication of animal experimentation but an extension of this and, of course, studies of speech function may only be carried out in man.

Proposed Course of the Project: It has been hoped for several years to eventually be able to attack directly the problem of the pathophysiology of motor activity itself. Equipment designed along the lines suggested by Dr. Lawrence Stark, formerly of M.I.T., for relating a visual input to a motor response has been laid aside in the past few years. This has not been due to any lack of interest but simply due to the lack of a person with an adequate engineering and computer background to devote sufficient time to the work in collaboration with the clinical service. The importance of quantitative motor studies for the evaluation of any therapeutic procedures is obvious. Perhaps more important, however, is that the reduction of the complexities of human movement to quantitative terms may permit model making and analysis according to more classical engineering techniques. It seems that a more basic approach to abnormal human movement is important if any advance in the basic understanding of this problem is to be achieved.

Honors and Awards: None

Publications: None

Serial No. NDS(I) - 56 SN/OC 304(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effect of Lesions Upon the Function and Structure of the Human Central Nervous System

Previous Serial Number: Same

Principal Investigator: J. M. Van Buren, M. D.

Other Investigators: F. P. Wirth, Jr., M. D. and R. C. Borke, B. S.

Cooperating Units: None

Man Years

Total:	2.1
Professional:	1.1
Other:	1.0

Project Description:

Objective: This project is directed toward the study of basic neuroanatomy and neurophysiology in man, making use of pathological material and the opportunities for study afforded by the operative treatment of neurological disease.

Methods Employed:

Anatomical studies:

(1) Serial sections of human and animal brains in celloidin for myelin and Nissl's series.

(2) Section and staining of primate brains with Nauta technique for demonstration of degenerating pathways.

Major Findings:

(1) The principal investigator's energies have been absorbed almost entirely in the present work. During this year the 108 montages of 54 brains with surgical or vascular lesions have been completed. This entailed a complete drawing of every 100th section through the hemisphere and every 40th section through the thalamus. This was followed by photographic reduction of the approximately 4000 drawings, preparation of montages and rephotographing for the final illustrations. Some work on these drawings has been continued as

errors have been uncovered but the major part of this work is finished. After the development of a standard nomenclature, all of the original descriptions of the montages were rewritten to incorporate this. Complete anatomical summaries using abbreviated terms were prepared.

Bibliographic research has been most time consuming since the investigator wished to personally make a complete survey of the literature at least as available in English, French and German. Although the major emphasis has been on man and primates, references in carnivores have also been covered. To date this has amounted to the reading and abstraction of some 700 articles and the preparation of a cross index of this material. More material is constantly uncovered.

To facilitate the study of the newly obtained pathological material (the 54 cases mentioned above), the codings of the areas of cortical, subcortical and thalamic destruction were transferred to punch cards (the same also was done separately for areas of cortical, subcortical and thalamic degeneration). A separate alphabetical listing was made as an index to facilitate study.

The material was submitted to Mrs. Sadowsky some months ago for preparation of positive and negative correlations, again to facilitate the study of this extensive material. To date these have not been forthcoming although they are promised soon.

In the meantime the study of the cerebrothalamic relationships of three major areas has been completed:

(a) The midline nuclei. Although these nuclei are much reduced and difficult to distinguish in man, the following have been seen with reasonable reliability: n. parataenialis, n. paramedianus anterior and posterior, n. habenularis medialis and lateralis and, if the massa intermedia is present, the n. commissuralis and n. endymalis. Contrary to current opinion, careful study of the present cases has demonstrated that these nuclei indeed degenerate with cerebral lesions. It is likely that the use of human material with long survival times has brought into play the factor of retrograde or anterograde transynaptic or double transynaptic degeneration. It is not possible to be certain of a topographical relationship due to the presence of only four cases, but definite degeneration has been seen in the n. paramedianus anterior, the n. commissuralis with extension into the n. centralis lateralis and in the n. parataenialis. A careful review of the literature has demonstrated that this has been reported a few times in experimental animals although it is not generally recognized.

(b) The anterodorsal group. The considerable material in the present series has allowed quite a good topographical relationship between the cingulate gyrus and the anterior nuclei to be worked out. It is

unlikely that the anterior nuclei project posterior to the paracentral lobule and that the n. dorsalis superficialis (n. lateralis dorsalis) projects to the posterior cingulate region. In the present material it was not possible to work out the region of projection of the n. anterodorsalis although there is reasonable evidence in man and primates reported in the literature.

An apparently new finding has been that of degeneration in the n. dorsalis superficialis following posterior hippocampal infarctions in five hemispheres. This is a rather unique lesion which as far as the investigator is aware has not been reproduced in animals due to the surgical inaccessibility of the area or, if it is reached, to the necessity of damaging adjacent structures extensively. No extensive documentation of this has ever been made in the literature although passing mention has been found of similar results in primates by two previous investigators. This matter is discussed more fully below.

(c) The medial and intralaminar region. This group includes the n. medialis, the n. intralamellaris and the centralis-parafascicularis complex. Degeneration in the n. medialis (n. medialis dorsalis) has been found in 34 cases so that the cerebrothalamic topographical relationships could be studied in considerable detail. The anteromedial portion of the n. medialis is related to the posterior orbital surface but the frontal pole probably lacks a significant projection to n. medialis. The frontal pole of n. medialis projects to the anterior second frontal convolution and the anterolateral region to the frontal opercular area. Working posteromedial along the frontal cortex the degeneration in n. medialis moves superoposteromedially and the posterior portion of the first frontal gyrus projects to the posterior pole of the n. medialis. Due to the lack of specific lesions in this area, it has not been possible to determine whether n. medialis may also project to the paracentral lobule. No evidence supports projection to the insular, temporal or parietal regions as has been suggested in some of the literature. It does, however, have a reasonably well defined projection to the precentral gyrus again as opposed to some negative findings in the literature.

The n. centralis (centrum medianum) was degenerated in ten cases and in all the striatum was damaged. The topographical relationships have not been as clear-cut as in the n. medialis but it seems likely that the anteromedial portion of n. centralis including the mediocellular part projects to the anterior medial putamen with the anterior pole of the nucleus projecting to the region of the junction of the anterior middle third of the putamen. Moving the lesion posterior along the body of the putamen moves the degeneration in the n. centralis laterally and posteriorly. It seems unlikely that the caudate head has any large projection to the n. centralis. This, however, is based on negative rather than positive evidence in that

cases with gross sparing of the caudate head and massive loss in the putamen have still permitted near complete degeneration of n. centralis. Unfortunately we have lacked specific lesions of the caudate head.

There were four cases of degeneration in n. parafascicularis. In all there was associated degeneration in n. centralis and damage to the putamen. Two of the cases had additional damage to the caudate head. It was not possible to draw further conclusions.

There was specific degeneration in the n. intralamellaris (intralaminar nuclei) in nine cases. This was regularly associated with cell loss in adjacent regions inside and outside of the internal medullary lamina. All cases of damage lay in the frontal lobe and no loss in this nucleus was seen with extrafrontal lesions. The point-to-point relationships in the frontal lobe precisely followed the relationships defined for the n. medialis.

(2) Anatomical studies of cases with loss of recent memory and spatial disorientation. Five hemispheres in three individuals have been available who, although showing a complicated neurological picture which varied somewhat from individual to individual, all demonstrated loss of recent memory and sufficient spatial disorientation so that they were at least unable to find their way around their own neighborhood and in two of the three cases required institutional confinement because of this disability. In all three cases a specific infarction, apparently of a branch of the posterior cerebral artery, destroyed the posterior portion of the hippocampus. Although lesions in the hippocampal gyrus, lingual gyrus and small extensions to the inferolateral pulvinar and n. ventralis caudalis were present, these were inconstant. In all material the fornix showed extensive degeneration and demyelination throughout its length. In one particularly favorably stained case it was possible to follow the demyelinated and heavily gliosed bundles from the descending branch of the fornix upward and backward to enter the stratum zonale of the thalamus anteromedial to the anterior nuclei and also in the myelinated band between the anterior nuclei and the subjacent thalamus. This then could be followed backward to the n. dorsalis superficialis which in all cases showed extensive degeneration.

This anatomical material has been interesting in demonstrating that the n. dorsalis superficialis (n. lateralis dorsalis) is also part of the limbic system and in demonstrating its relationship to the fornix which heretofore has not been studied. The coincidence of spatial disorientation and recent memory in this relatively extensive material has re-emphasized the importance of the hippocampus and its central connections in the recording of newly acquired material.

Significance to Bio-medical Research and the Program of the Institute:

(1) The present studies, although incomplete, have vindicated the original contention that the serial section study of a relatively large number of human cerebri (54) with focal, vascular or surgical lesions would shed new light upon the corticothalamic relationships in man. For this material the investigator is deeply indebted to Dr. Paul Yakovlev for the use of the collection amassed by him over 40 years. It is unlikely that a collection of this type will ever be prepared again so that the present work is in response to what is probably a unique situation.

At present the collection is being removed from the Harvard Medical School to temporary shelter due to lack of funding. Dr. Yakovlev is much concerned about the future of his collection and would welcome an offer to permanently house this in an area where it would be available for use and where it would continue to be expanded. The entirely unique nature of this collection of over 1,000,000 slides comprising serially sectioned embryo, fetal, developmental defects and focal lesions in man cannot be over estimated as a National Resource. As an addition to N.I.N.D.S. it would permit continued studies in depth of the human nervous system.

The opportunity to make an extensive review of the literature of the thalamus particularly in primates has uncovered a large number of inconsistencies and contradictions which have opened many avenues for future anatomical research. These inconsistencies are not apparent in current textbooks which give a rather finished appearance to the subject of the cerebrothalamic connections. For this reason the extensive time devoted to the bibliographic research has appeared justified.

(2) Totally apart from its academic importance, the present work will form the basis for future studies in physiology of the human thalamus. Indeed, without this knowledge of the relationship of the thalamic nuclei to the x-ray landmarks in the third ventricle, no meaningful physiological research in deep structures in man can be carried out. The many fallacies apparent in the current literature attest to the need for these studies.

Proposed Course of Project:

(1) Due to the controversy regarding the central thalamic connections of the insula, Dr. Fremont Wirth is undertaking ablation of the insula with varying surgical approaches through the opercula. One hemisphere is for Nissl and myelin studies with a 60 day degeneration period and the other is for Nauta studies to show afferent fibers to the thalamus. He has now placed the lesions in six animals and the histological material will be available for study when he enters the investigator's laboratory full time.

(2) The plans for the thalamic atlas are as follows: The cerebrothalamic relationships of the lateral portion of the thalamus including the n. lateralis polaris, n. dorsalis oralis, n. dorsalis caudalis, n. ventralis

oralis externa and interna, n. ventralis caudalis externa and interna and n. ventralis caudalis parvocellularis remain to be studied as does the posterior portion of the thalamus including the n. pulvinaris and the corpus geniculatum mediale and laterale. It is hoped that, when available, the positive and negative correlations will speed this aspect of the work.

(3) Following this study of connections the cytoarchitecture of the nuclei will be formally written up. At the present time all of the photographs of Golgi preparations in Miss Borke's study are available and as soon as the N.I.N.D.S. Photography Laboratory is operational she will begin work on the cytoarchitectural photographs of the individual nuclei in Nissl.

(4) The above studies (with indexing) will terminate the work on Volume I. The final step in completing Volume II will be the anatomical variation studies of the intrinsic thalamic nuclei. (The variation studies of the gross human diencephalon of 25 specimens in each of the three major planes has already been completed.) Every effort is being made to obtain a summer medical student to facilitate this work.

Honors and Awards: A medallion has been received from the College of Physicians and Surgeons with a citation as follows: "On the occasion of the 200th anniversary of the founding of the Medical School of King's College, the College of Physicians and Surgeons, Columbia University, is proud to present this commemorative medallion to John Van Buren in recognition of his achievements which have contributed to the stature of the University." Signed H. Houston Merritt, Dean, and Grayson Kirk, President.

Publications:

Van Buren, J. M. and Borke, R. C.: Alterations in speech and the pulvinar. Brain, in press.

Serial No. NDS(I) - 62 SN/OC 906(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Effects of Cold and the Relation of Temperature to Functions of the Central Nervous System

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, F.R.C.S., F.A.C.S.

Other Investigators: None

Cooperating Units: None

Due to lack of technical support this investigation has been discontinued.

Serial No. NDS(I) - 62 SN/OC 907(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Trauma to the Nervous System. Experimental and Clinical.

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, F.R.C.S., F.A.C.S.

Other Investigators: F. Fisch, Ph.D., R. M. Mahone, M.S., E. Hendler, Ph.D., P. Corrao, M.D., D. Sass, M.D., F. Letcher, M.D., L. C. Parsons, Ph.D., K. M. Nelson, M.D., R. Naumann, M.D., R. Grubb, M.D., H. Metz, M.S.

Cooperating Units: Personnel Protection Branch, Naval Ship Research and Development Center; Air Crew Equipment Laboratory, Navy Ship Yard, Philadelphia, Pennsylvania; Laboratory of Biophysics, Naval Medical Research Institute; Division of Research Services, Biomedical Engineering and Instrumentation Branch, NIH.

Man Years

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objective: A clinical and experimental study of the biophysics, physiology and pathology of trauma and its effects on the central nervous system in order to enable prediction of the results of such trauma in man and to establish its management on a rational basis.

Methods Employed:

1. The Field Emission type of ultra-high-speed x-ray apparatus is now in use and preliminary experiments with carotid angiography and radiopaque markers are in progress.
2. Lexan calvaria are being prepared and studied by high-speed cinematography during impact for measuring actual brain displacements.
3. The arterial and C.S.F., pO_2, pCO_2, and pH are being measured simultaneously in patients after severe head injuries and in monkeys before, during and after experimental concussion.

4. An apparatus able to provide controlled degrees of impulsive loading ("whiplash") to the animal without direct head impact has been constructed and is now in use to supplement information previously obtained from our head impact device.

5. A hyperbaric chamber is being fitted with a "concussion gun" to enable the study of experimental head injury and its effect under controlled conditions of atmospheric composition and pressure.

6. A technique for chronic implantation and recording from cortical and deeper brain structures via fine stainless steel and platinum electrodes is being developed. Four monkeys have been prepared with a total of 8 bipolar depth electrodes and 6 unipolar surface electrodes in each animal. Electrophysiological studies (slow wave phenomena as well as evoked potentials and extracellular "neuronal pools") will be recorded before and after experimental head injury. Television techniques have been developed for monitoring monkey behavior and EEG simultaneously with particular reference to sleep disturbances after trauma.

7. Modified Florey-Barr brain observation chambers have been developed. These will allow the administration of precise degrees of focal cortical trauma while allowing simultaneous visual (cinematographic) observations as well as measurement of gas tensions in the C.S.F. overlying the damaged area and concurrent recording and of EEG and D.C. potentials.

8. Isotope cisternography and ventriculography are being used to diagnose occult hydrocephalus, a late sequela of severe head injuries. The relationship between this complication and post-traumatic epilepsy is also being investigated in selected patients.

9. The silicone rubber cerebrospinal fluid reservoir is being used to record intraventricular pressures over long periods of time in selected patients with severe brain trauma.

10. Experiments using a latex balloon have enabled the determination of the elastic modulus of brain in vivo and after death.

Major Findings:

1. The natural frequency of brain rotation as measured by the radiographic and lexan calvarium techniques is between 5 to 10 cycles per second (in the monkey).

2. Our prediction of the injurious effects of a whiplash injury has been confirmed in over 80 monkeys. In addition to defining the levels of rotational velocity and rotational acceleration required for concussion, we have demonstrated the regular production of surface brain hemorrhages (mainly subdural) by whiplash injury. It should be emphasized that surface hemorrhages are extremely rarely obtained by direct head impact injury in monkeys (short of skull fracture). The level of rotational velocity at which concussion is produced in 50% of monkeys is about 50% greater than that required for concussion produced by occipital head impact indicating the significance of local effects.

3. Knowing the rotational frequency of brain as well as the concussive short-duration rotational velocity thresholds for head impact and

whiplash injury we can calculate the long-duration rotational acceleration thresholds and define tolerance curves based on the analogy to a mass-spring system.

4. Our scaling method dependent on an inverse 2/3 relationship of brain mass is being tested to extrapolate experimental data for energy thresholds for cerebral concussion in lower primates to thresholds for cerebral concussion in man. This is based on the equation $R = \frac{c}{m^{2/3}}$ where R = rotational acceleration required for injury threshold; m = brain mass in grams and c = a constant derived from our experimental data. At the present time we are obtaining experimental data for the squirrel monkey (brain wt. = 30 gm), and for the chimpanzee (brain wt. = 400 gm). Extrapolation to the human brain weight will enable us to obtain an indication of the injury threshold for concussion in man (in terms of angular head velocity and acceleration). Final confirmation of this prediction will be sought from a careful analysis of accident data in man. To date the experimental data from the chimpanzee and squirrel monkey are displaying excellent fit to the predicted levels.

5. In studies on C.S.F. O_2 tensions, we have determined the dynamic relationship between pO_2 in this fluid with relation to the pO_2 of arterial blood in the monkey. We have also shown a species difference in the response of cortical tissue pO_2 to hyperoxygenation. Thus in monkeys, increased serum pO_2 is associated with a fall in cortical pO_2, while in rats a rise in brain pO_2 is seen. The implications of these observations for brain vascular autoregulatory activities in the more highly evolved species are worthy of further investigation. Current experiments are revealing the serum-C.S.F. pH and gas tension relationships after varying grades of CNS trauma.

6. Occult hydrocephalus is a fairly common occurrence after severe closed head injury in man and alleviation by ventriculo-jugular shunting can result in significant clinical improvement as late as three years after apparent stasis in the neurological/intellectual deficits. A total of thirty patients have been studied to date. The data would suggest that only those patients showing stasis of isotope in the C.S.F. after isotope cisternography will improve after shunting. This hypothesis is now under test and if proved correct will provide a useful guide to selection of such patients for surgery.

7. Two well documented cases of post-whiplash subclinical hematomas have come to our attention. One of these was fatal in a middle-aged woman as early as three days after the whiplash injury and the other necessitated surgical removal of the clots. These clinical experiences and our experimental results have enabled us to advise physicians to treat severe whiplash injuries not only from the musculoskeletal and cervical nerve root aspects but also with the realization that cerebral concussion and other sequelae of a closed head injury may have occurred.

8. The elastic modulus of brain = .5 to 3.5 x 10^5 dynes/cm^2 (increasing with strain). This modulus changes slightly after death and markedly after formalin perfusion.

9. Pupillary dilatation after head injury is found to be a useful index of severity of injury.

Significance to Bio-medical Research and the Program of the Institute:

1. The ability to define an injury threshold for cerebral concussion and other effects of nervous system trauma in man suggests the possibility of a more adequate definition of performance criteria for protection against head injury and whiplash injury in automobile accidents and motorcycle crashes. This will include the design of helmets of all types as well as automobile interior and exterior design. It is for this reason that our research is being supported by the Department of Transportation as well as the U. S. Navy.

2. The finding that a cervical collar raises the threshold for experimental cerebral concussion in the monkey suggest a number of ways in which protection against such injury may be achieved under a variety of conditions. Currently an inflatable collar device and an inertia strap for helmet restraint are being tested at the Air Crew Equipment Laboratory in Philadelphia.

3. The findings that whiplash injury alone can produce significant degrees of visible brain surface hemorrhages raise the issue of such lesions being produced by whiplash trauma in man (as commonly occurs after rear-end automobile collisions). Further work is required to confirm these observations.

4. The finding of definitive alterations in sleep patterns after head injury has important implications in the understanding of the post-traumatic syndrome.

Proposed Course:

1. Biomechanical studies will be continued on fresh in vitro and in vivo samples of nervous tissues to establish fundamental data on the viscoelastic properties of these tissues and their behavior under stress.

2. Input from the above and from the ongoing mechanical, biochemical, and physiological studies will be used to develop the theoretical analysis and produce a theoretical model for the behavior of the brain in response to trauma.

3. Controlled "whiplash" and whole-body acceleration trauma experiments in monkeys will be conducted to round out other aspects of the mechanics of injury in order to complete the definition of thresholds of CNS injury.

4. Prophylactic and therapeutic measures aimed at reducing the noxious and enhancing the beneficial responses after trauma will be studied experimentally in the monkey.

5. Detailed neuropathological studies are now being conducted with Dr. P. Corrao in the laboratory of Dr. Snodgrass at the Naval Medical Research Institute.

6. Experimental verification of our scaling method ($R = \frac{c}{m^{2/3}}$) for both impact and whiplash trauma using the squirrel monkey and chimpanzee as well as the rhesus will then allow testing of the prediction of concussive threshold for man against available accident data.

7. The neurophysiologic effects of head injury will be studied in an attempt to define further the mechanism and sequelae of head injury.

Honors and Awards: None

Publications:

Ommaya, A.K.: A critique of Hodgson's - Proof for the correlation of head impact tolerance and linear acceleration. In Conference Proceedings of the Impact Injury and Crash Protection Symposium, May 9-10, 1968, Wayne State University (In press).

Ommaya, A.K. and Corrao, P.: Pathologic biomechanics of central nervous system injury in head impact and whiplash trauma. In Proceedings of the International Conference on Accident Pathology, June 6-8, 1968, Washington, D.C., U. S. Government Printing Office (In press).

Yarnell, P. and Ommaya, A.K.: Experimental cerebral concussion in the rhesus monkey. Bull. N.Y. Acad. Med. 45: 39-45, 1969.

Hirsch, A.E., Ommaya, A.K., and Mahone, R.M.: Tolerance of subhuman primate brain to cerebral concussion. Dept. Navy, Naval Ship Research and Development Center, Report 2876, Washington, D.C., 1968, 19 pp.

Mahone, R.M., Corrao, P., Hendler, E., Shulman, M., and Ommaya, A.K.: A theory on the mechanics of whiplash produced concussion in primates. J. Biomechanics (In press).

Metz, H., McElhaney, J., and Ommaya, A.K.: A comparison of the elasticity of live, dead, and fixed brain tissues. J. Biomechanics (In press).

Ratcheson, R.A. and Ommaya, A.K.: Experience with the subcutaneous cerebrospinal-fluid reservoir. New Eng. J. Med. 279: 1025-1031, 1968.

Serial No. NDS(I)-62 SN/OC 913(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Single Cell Discharges from Various Nervous Structures and Their Functional Organization in Particular Reference to Somatosensory Activity in Man

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M.D., Ph.D., John Van Buren, M.D., Ph.D.

Other Investigators: Eugene Harris, Ph.D., T.C. Chen, Ph.D., Raymond Mejia, B.S.

Cooperating Units: CR-LAS; N-OB; CR-DSA

Man Years:
Total: .6
Professional: .3
Other: .3

Project Description:

Objectives:

a. To extend the experimental observations from laboratory studies to clinical surgical procedures in order to gain further understanding of motor function in man.
b. In addition, responses of cells in various brain structures to visual, tactile, and auditory stimulation are investigated.

Methods Employed:

a. Multiple electrodes are placed in the motor cortex, thalamus, caudate nucleus and pulvinar. Electrodes are also placed in the extensor and flexor muscles and on the surface of motor cortex.
b. Extracellular action potentials from these structures are simultaneously recorded before, during, and after, the voluntary and involuntary movements of patients subject to operations.
c. Responses to tactile, visual, and auditory stimulation are also recorded. This datum is stored in magnetic tapes and is to be converted into digital signals, ready for computer analysis.

Major Findings: At the time of this report the investigators are still involved in the technological refinements, and no conclusive statements can be made.

Significance to Bio-medical Research and Program of the Institute: This study will add to our understanding of motor function or dysfunction in man and eventually provide benefits to patients with motor disorders.

Proposed Course of Project: This is a continuation of the project titled: Single Cell Discharges from Motor Cortex and Basal Structures of Man. It will be continued further and eventually extended to the study of the cerebellum.

Honors and Awards: None

Publications: None

Serial No. NDS(I) - 63 SN/OC 1025(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Tumors of the Nervous System

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, F.R.C.S., F.A.C.S.

Other Investigators: P. Paoletti, M.D., R. Paoletti, M.D., F. P. Wirth, M.D. and H. Kaufman, M.D.

Cooperating Units: Neurosurgical Clinic and Institute of Pharmacology, University of Milan, Italy

Man Years

Total:	.8
Professional:	.4
Other:	.4

Project Description:

Objective: To understand how gliomas develop and grow and how best they can be treated in man.

Methods Employed:

1. A special silicone device described as a cerebrospinal fluid reservoir has been designed by the principal investigator. This allows sterile access to the ventricular cerebrospinal fluid by subcutaneous puncture. This device allows utilization of the cerebrospinal fluid pathways for introduction of cytotoxic agents for chemotherapy, either by infusion or perfusion.

2. Estimation of desmosterol in the cerebrospinal fluid has been shown by the Paolettis to be a dependable way of indicating the presence of gliomas, particularly after preliminary blockage of cholesterol synthesis with Triparanol in patients with this type of brain tumors only. Arrangements have been made to freeze samples of cerebrospinal fluid from all patients admitted to this project. Frozen samples are sent for estimations of desmosterol to the Paolettis in Milan with the object of testing three hypotheses:

a. that desmosterol levels in the cerebrospinal fluid accurately indicate the presence of gliomas;

b. that higher levels of desmosterol will occur at times of accelerated growth of the tumor and that these times will be used to determine timing of therapy;
c. that the established desmosterol levels will accurately reflect the destructive effect of 8-Azaguanine on glioblastoma multiforme and will thus serve as a suitable index for dosage and duration of chemotherapy.

Major Findings: Because of the inadequate collaboration that extended from the National Cancer Institute as detailed in last year's report, it was decided to terminate that arrangement and continue this project as an entirely Surgical Neurology Branch/NINDS one. Extreme scarcity of beds remains a problem. Temporary palliation was provided by the use of two beds from the National Institute of Mental Health. This arrangement was the result of exploring possible collaborative efforts on the biogenic amines in the cerebrospinal fluid with Dr. I. Kopin and on the relationship between cerebrospinal fluid pressures and sleep mechanisms with Dr. Snyder in such patients. These arrangements had to be terminated because of procedural problems.

A review of the two years of intrathecal chemotherapy involving eighteen patients with glioblastoma has shown that the first drug tested, Methotrexate, is not a useful agent to use as an intrathecal drug against gliomas; sixteen of those eighteen patients are dead. The two survivors have been retreated with 8-Azaguanine, the current drug under test. In one patient a previously obvious mass seen on pneumoencephalography has vanished and he remained well until three years after the initial diagnosis when he committed suicide. Three other patients are currently undergoing treatment with 8-Azaguanine.

Aspects of this research have been reported in a publication listed under Project Serial No. NDS(I) - 56 SN/OC 304(c). A further publication is in preparation.

Significance to Bio-medical Research and the Program of the Institute: It is hoped to improve the presently inadequate treatment of glial tumors of the nervous system, and hopefully extend clinical cures to patients with glioblastoma multiforme and other malignant gliomas.

Proposed Course: To continue investigation of patients with glioblastoma along the lines indicated in order to determine the validity of the hypotheses outlined above. It must be stressed however that the current rate of progress of this study is quite inadequate due to inavailability of hospital beds. The utilization of only one bed means that only two or three patients can be studied per year.

Honors and Awards: None

Publications:

Ommaya, A.K.: Chemotherapy of gliomas. Transactions & Studies of the College of Physicians of Philadelphia. 36: 119-120, 1968.

Serial No. NDS(I)-65 SN/OC 1206(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Microbial Characteristics in a Neurosurgical Environment

Previous Serial Number: Same

Principal Investigator: Maitland Baldwin, M.D.

Other Investigators: L. G. Herman, Ph.D., Edward Rich, Ph.D.
F. Lamberti, R.N., Delta Trickett, R.N.

Cooperating Units: R-ES; CC-EHS

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: Analysis of salient microbial features in a neurosurgical room environment.

Methods Employed:

a. Personnel and environmental microbial sampling.
b. Analysis of physical environment.
c. Analysis of personnel structure.
d. Analysis of laminar flow system and comparison with conventional system.

Major Findings: This year's work was concerned with testing of portable laminar flow systems in relationship to operating table and the wheeled stretcher. In addition, the routine microbial sampling of the neurosurgical theaters was carried out. The laminar flow air unit used is a prototype manufactured by Air Control, Inc. Three air velocities are provided as step functions producing velocities of 30, 50 and 80 feet per minute average at mattress or cover level. Lighting is provided for reading or examination. There is no temperature control or provision. A normal complement of pre- and absolute filters is used. Canopy filter size is 42 x 56 inches. Using Rodac plate impressions of walls, floors, linen, countertops, etc., acceptable time bases were scheduled so that such samplers could reflect the microbial environment when the air was not specifically moved as in the three velocities mentioned above. Test periods were conducted at approximately two hour intervals during which four operating room personnel followed a simulated

routine of preparation and administration of anesthesia. The opening of doors, closets, packages, moving of equipment, etc., simulated the real task. In this situation, it seemed clear that particles being shed by attending personnel were collected by samplers outside of the air stream. Obviously, the high air flow area (under the laminar flow) provides fewer organisms than the surrounding ambient, despite the fact that four people with exposed faces, hands and wrists were working during test periods within the laminar column. It is also clear that particles shedding from skin of personnel working within the laminar area were externalized outside the configuration but added to the ambient. This may be indicative that laminar flow used in the single modular configurations should not be adjacent to an otherwise unprotected patient outside of such a configuration, as he may well receive reflected particles containing microbial contaminants. It seems that the filtered moving air provided protection of the patient, and the continuous filtered circulation cleans the room almost as fast as it is contaminated by the attending workers.

Significance to Bio-medical Research and Program of the Institute: This is a further experimental examination of laminar flow equipment in a real life situation and demonstrates not only feasibility but desirability. It would seem that this type of modular equipment may have considerable application in smaller operating rooms where wall size laminar flow configurations are impractical and uneconomical, and also in emergency rooms or in isolation rooms, particularly where burn cases are to be treated.

Proposed Course of the Project: We will continue the testing on these prototypes now in the operating room and in a nursing unit environment.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-66 SN/OC 1245(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: EEG Learning Correlates using Scalp and Intracranial Depth Electrodes.

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D., William H. Sheriff, M.A., and M. Buchsbaum, M.D.

Other Investigators: J. Van Buren, M.D., Ph.D., M. Baldwin, M.D., A. Ommaya, F.R.C.S., J. Bryan, M.A., and M. Haddox, R.N.

Cooperating Units: Laboratory of Psychology, NIMH

Man Years
Total: .2
Professional: .1
Other: .1

Project Description:

Objective: To study left and right hemispheric contribution to perception, information storage and retrieval of different types of material.

Methods Employed: Two classes of stimuli, verbal (words) and nonverbal (nonsense patterns or dot arrays) were randomly delivered for rapid, binocular viewing. Average evoked cortical responses to the discriminanda were calculated simultaneously from left and right occipital EEG tracings in a group of normal subjects. The LINC computer was used to generate the perceptual stimuli. A-D conversion of bipolar EEG activity and the computation of the evoked responses were performed by the LINC; the entire test session and signal averaging was conducted on-line.

Averaged evoked potentials during learning of nonverbal, temporal sequences on a spatial maze were analyzed. The task requires that the subjects traverse a predetermined path from a start to a goal position with a special stylus. During performance, correct and incorrect selection of maze points are signaled to the subject by two distinct tones. Prior to testing, the same tones were presented as neutral stimuli in order to establish basal EEG responsiveness. The signal and EEG events are recorded on an Ampex magnetic recorder for subsequent, off-line analysis by the LINC computer.

Major Findings: Visual discrimination of verbal and nonverbal stimuli is accompanied by dissimilar evoked potentials from the left and right occipital lobe. Word and dot arrays evoke greater differences in averaged responses from the left hemisphere whereas the right hemisphere reflects greater differences during perception of word and design stimuli. In each instance, the interhemispheric differences are manifested in the shape and latency of the evoked potentials. The findings extend the observations that the two cerebral hemispheres in man which govern verbal and nonverbal behavior, display distinctive, intrinsic electrographic properties.

Maze performance by patients with multiple-contact electrodes situated in the thalamus (200(c)) or cingulum (1424(c)) yielded differential evoked responses which correspond to correct or incorrect performance. Evoked responses recorded from the right pulvinar showed distinct waveform, most notably for incorrect selections. No evoked pattern was evident from other sites in the right thalamus or caudate region. In one patient with bilateral cingulum electrodes, an evoked response was elicited from both cinguli, again, in association with error performance. In each instance, the evoked response accompanying correct performance was less distinct than that for incorrect responses.

Significance to Bio-medical Research and the Program of the Institute: Behavioral data available from patients following unilateral temporal lobectomy reveal significant perceptual and learning deficits, dependent upon the verbal or nonverbal nature of the material. The technique employed in this project affords a more precise method for examining participation by cortical and subcortical structures during the course of perception and registration.

Proposed Course of Project: To develop additional intellectual tasks and programs for EEG data analysis with wider application to patients with different brain lesions.

Honors and Awards: None

Publications: See Project M-P-C-(C)-36

Serial No. NDS(I)-67 SN/OC 1417(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Changes in Physiological Properties of Brain Tissue at Low Temperatures and in Other Pathological Conditions

Previous Serial Number: SAME

Principal Investigators: Choh-luh Li, M.D., Ph.D., Igor Klatzo, M.D., Joseph Fenstermacher, Ph.D.

Other Investigators: None

Cooperating Units: C-LCP

Man Years:
Total: .2
Professional: .2
Other: .0

Project Description:

Objective: This is a continuation of a project reported last year and an extension to study the extracellular space of the gray matter in the brain. Changes in electrophysiological properties and histological manifestations of the brain tissue under various conditions are also under investigation.

Methods Employed:

a. Cats under Fluothane anesthesia are used.
b. Some of the animals are subject to hypothermia and the others serve as controls.
c. Action potentials and resting potentials are recorded from single nerve cells in the cerebral cortex, specific resistivity of the cortex and white matter is determined, spontaneous electrical activity and transcallosal evoked responses are registered. These data are simultaneously obtained at a given temperature, which is continuously measured from the cortex and white matter with thermisters; they are stored in magnetic tapes or photographic film to be analyzed by either a Linc computer or manual measurements.
d. The extracellular volume at temperatures of 36°C and 15°C

is determined by the concentration profile of radio-active inulin in the periventricular gray matter. The radio-active inulin is perfused into the lateral ventricle through a small cannula and is recovered from the posterior fossa. The radio-active perfusate introduced and collected, as well as any possible amount of radio-active substance in the general blood circulation, is also quantitatively determined.

e. Experimental brain edema is produced by injection of liquid paraffin or peanut oil into the external carotid artery. In some animals it is also produced by injection of Triethyltin.
f. Experimental hypertension is produced and the change in the blood pressure and electrical activity of the cortex is continuously recorded by a polygraph.
g. The brain is removed at the end of the experiment and is examined with various histological techniques and, in some cases, with electronmicroscopy.

Major Findings:

a. In normothermic animals the extracellular volume of the gray matter of the brain is 13.5% of the total volume, when it is determined by the electrophysiological method, and is 13.8% by the inulin concentration profile method. At low temperatures the study has not been computed, and a conclusive statement cannot be made at this time; although the electronmicroscopic study appears to indicate that there is no change in the extracellular space.
b. In hypothermic animals the resting potentials of the cortical neurons are unchanged. However, in the process of cooling the frequency of single nerve cell discharges shows an increase and decrease and finally complete disappearance at 20°C. This phenomenon also occurs in the spontaneous electrical activity and evoked potentials recorded from the cortical surface. In hypothermic animals there is an inhibition of passive transport of radio-active inulin and sucrose from the ventricle to the extracellular compartment.
c. Electrophysiological measurements conclusively differentiate extracellular from intracellular edema and prove to be valuable in the collaborative investigation of histopathological tissue of the brain.

Significance to Bio-medical Research and Program of the Institute: The electrophysiological study indicates that there is no swelling of nerve cells in the brain, even when the brain temperature is lowered to 12°C. This gives assurance to our patients, who are subject to hypothermia, if the hypothermia procedure is properly carried out.

It is of considerable importance that at normal body temperature the volume of the extracellular compartment is found to be consistent, as it is determined by two entirely different methods. The value thus obtained is slightly larger than those reported by neurochemists. In all cases, however, skepticism has been raised by biophysicists. The present study serves as a stimulation for further and more accurate determination of extracellular space which is believed to be of importance in the understanding of brain metabolism.

Proposed Course of Project: It is planned that a collaborative effort will be made with Dr. Kenneth S. Cole to determine the extracellular volume of brain tissue.

Honors and Awards: None

Publications: None

Serial No. NDS(I) - 67 SN/OC 1418(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Surgical Treatment of Arteriovenous Aneurysms (Malformations) of the Spinal Cord

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, F.R.C.S., F.A.C.S.

Other Investigators: G. Di Chiro, M.D., J. Doppman, M.D.

Cooperating Units: Section of Neuroradiology, Medical Neurology, NINDS; Diagnostic X-ray Department, Clinical Center, NIH

Man Years

Total:	.6
Professional:	.3
Other:	.3

Project Description:

Objective: To establish the pathological anatomy and natural history of spinal arteriovenous malformations in order to provide the best surgical treatment for such lesions in man.

Methods Employed: Selective aortographic techniques for radiologic demonstration of the arterial blood supply of such lesions have been developed by Drs. John Doppman and Giovanni Di Chiro (see Individual Project Reports by these investigators on diagnostic aspects of this project) based on the experience of Djinjian, Faure and their co-workers in Paris, France. These workers had applied the technique of extradural and often extraspinal ligation of arterial blood supply of such lesions. We have investigated the effect of intradural ligation of such vessels "feeding" the malformation in fifteen patients.

Major Findings: Twenty-seven patients with this disease have been studied to date. Once the arterial supply is clearly demonstrated, usually by the selective catheterization of the intercostal or lumbar aortic branch from which the vessel arises, the surgical technique for exposure and intradural ligation of the abnormal blood supply is now well established by our experience. Extremely dramatic improvement in neurological deficits has

occurred in at least 8 patients, the others improving either slightly or showing arrest in an otherwise progressive neurological deficit. Not a single patient has been made worse by this procedure. This compares very favorably with any other form of treatment and offers hope of permanent relief since a follow-up of nearly three years is now available for our first three patients. It has also been noted that the shorter the duration of symptoms and neurological deficit, the more rapid and complete is the dissipation of such deficit after this type of surgery.

Aspects of this research have been reported in publications listed under Project Serial No. NDS(I) - 65 MN/NR 1195(c) and CC-26.

Significance to Bio-medical Research and the Program of the Institute: In addition to providing a solution to this clinical problem, our surgical method and diagnostic studies provide an opportunity to contribute significantly to the knowledge of the normal and abnormal blood supply of the human spinal cord as well as to related aspects of spinal cord physiology and pathology.

Proposed Course: To obtain a large series of such patients and follow them for a significantly long period of time in order to answer the intentions of the objectives stated for this project.

Honors and Awards: None

Publications:

Ommaya, A.K., Di Chiro, G., and Doppman, J.: Ligation of arterial supply in the treatment of spinal cord arteriovenous malformations. J. Neurosurg. (In press).

Serial No. NDS(I)-67 SN/OC 1424(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Response Modulation by the Limbic System in Man: Neuropsychological and Physiological Changes with Amygdaloid and Cingulate Lesions.

Previous Serial Number: SAME

Principal Investigators: Paul Fedio, Ph.D. and Ayub Ommaya, F.R.C.S.

Other Investigators: Theodore Zahn, Ph.D., William Sheriff, M.A. and M. Haddox, R.N.

Cooperating Units: Laboratory of Psychology, NIMH

Man Years
Total: .2
Professional: .2
Other: 0

Project Description:

Objective: Various physiological and electrophysiological changes in cognitive and emotional functions are studied in relation to limbic structures in man.

Methods Employed: Therapeutic benefits from bilateral cingulumotomy in reducing 'appreciation of pain' are examined by standard psychophysiologic and behavioral measures prior to and following surgery. Performance on perceptual and short term memory tasks is also studied during electrical stimulation of chronic electrodes implanted in the limbic system.

Major Findings: For 5 patients with bilateral cingulum electrodes, stimulation of the left cingulum was associated with marked impairment in memory for verbal memoranda. No comparable changes in memory accuracy followed right cingulum stimulation. Whereas stimulation in the left thalamus, specifically the pulvinar, produced anomia [200(c)], cingulum stimulation failed to modify naming behavior. One patient with an electrode directed toward the right amygdala showed no change in name-recall performance during stimulation.

Preoperative and postoperative physiologic evaluation of patients with intractable pain showed a marked decrease in 4 cases in electrodermal activity

following cingulumotomy. In the remaining cases, 1 patient showed a moderate GSR decrease, 1 patient showed no change. These measures also appear to be related to independent observations regarding changes in pain perception following therapeutic coagulation.

Significance to Bio-medical Research and the Program of the Institute: This project provides an opportunity to define precise behavioral and autonomic concomitants of limbic functions in man. The observations contribute to a better understanding of psychophysiologic mechanisms related to modifications in pain apperception following cingulumotomy in patients with intractable pain.

Proposed Course of Project: To continue the study and to extend the protocol to include patients subject to temporal lobectomy.

Honors and Awards: None

Publications: None

Serial No. NDS(I) - 68 SN/OC 1522(c)
1. Surgical Neurology Branch
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Neural Control of Visuo-Motor Learning and Memory

Previous Serial Number: Same

Principal Investigator: A. K. Ommaya, F.R.C.S., F.A.C.S.

Other Investigators: J. Bossom, Ph.D., P. Fedio, Ph.D.

Cooperating Units: Primate Laboratory, Brooklyn College, New York

Man Years

Total:	.6
Professional:	.3
Other:	.3

Project Description:

Objectives:

1. To analyze the neural basis of prism adaptation as a tool to study visuo-motor mechanisms.
2. To determine the mutual roles of complexity and time in the mechanism of memory and develop a predictive theory of memory.
3. To ascertain the role of the limbic system in memory and visuo-motor learning.

Methods Employed:

1. Prism adaptation in monkeys combined with classical lesion analysis using behavioral techniques is the main technique. A test for reaching-grasping behavior has been developed by Dr. Joseph Bossom.
2. A matching from sample memory test capable of controlled variation of time and complexity of task has been developed.
3. Selected patients admitted for surgical relief of either pain or epilepsy often need depth electrodes implanted in either the cingulum or amygdala. Psychologic and memory tests before and after stimulation and coagulative electrode lesions at these sites provide important data for analysis. Stimulation of these structures at various parameters of current and frequency also are proving very rewarding.

Major Findings:

1. Bilateral dorsal rhizotomy in monkeys (C2 to T4) does not obliterate reaching-grasping and prism adaptation is unimpaired. This finding supported older theories of a "motor corrollary discharge" capable of providing sufficient "local feed-back" in the brain to perform useful motor acts. Lesions in the temporal, striate and parietal cortex do not impair this mechanism which is inhibited only by frontal lobe lesions (involving head of caudate nucleus or bilateral stereotaxic caudate nucleus lesions).

2. After unilateral dorsal rhizotomy in monkeys restraint of the normal arm allows use of deafferented arm for reaching and grasping.

3. The matching from sample memory test has been well tested in a group of 40 normal volunteers and scoring established. Testing of patients with surgical lobectomies is now in progress.

4. Cingulum stimulation impairs short-term memory much more when it is in the dominant hemisphere and also qualitatively more decisively when equivalent stimulation of the amygdala (right or left). Further observations have also confirmed non-verbal functional localization in the right hemisphere.

5. Unilateral stereotaxic amygdalotomy has produced significant improvement in behavior and seizures in a small group of patients.

Significance to Bio-medical Research and the Program of the Institute: It is hoped that the understanding of visuo-motor learning and memory will lead to a more accurate system of guiding the acquisition of neonatal and infant motor skills as well as improve the rationale and quality of rehabilitation after severe neurologic disease with crippling sequelae.

Proposed Course: To continue on the three aspects of the project, one of which is in collaboration with Dr. Bossom with whom the bulk of the behavioral testing in monkeys is carried out at the Brooklyn College, New York. Two reports are currently in preparation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1524(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electrical Resistivity of Brain Tissues

During FY 1969 this project was incorporated with Serial No. NDS(I)-68 SN/OC 1527(c).

Serial No. NDS(I)-68 SN/OC 1525(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electrical Resistivity of Biophysical Models

Previous Serial Number: SAME

Principal Investigators: Kenneth S. Cole, Ph.D., Choh-luh Li, M.D., Ph.D.

Other Investigators: Anthony F. Bak

Cooperating Units: N-LB; M-LNP

Man Years:
Total: .3
Professional: .2
Other: .1

Project Description:

Objective: To evaluate the applicability of the Maxwell Equation for estimating cellular volumes in suspensions consisting of artificial cells of different sizes and shapes in electrolyzed solutions.

Methods Employed:

a. Two electrodes are placed in a specially constructed container. The electrodes are made of stainless steel plates, triangular in shape, with a contact surface of approximately 18 sq. in. and a thickness of 0.002 in.
b. Between the two electrodes are suspensions consisting of different sized and shaped artificial cells in electrolyte solutions of various concentrations.
c. Alternating current is passed from one electrode to the other at frequencies of 0.1, 1.0 and 10.0 kilocycles per second.
d. The specific resistivity of the suspension between the two electrodes is measured by means of an impedance comparer. The temperature of the electrolyte solution of the suspension is measured.

Major Findings: Since this research project was initiated toward the end of 1967, a great deal of discussion and experimentation has been made in regard to the material and the technique employed. At this stage of

investigation no conclusive statement about the observations can be made. However, it is apparent that this model can be used for the evaluation of the Maxwell Equation and its application to the study of extracellular space of the nervous tissues under different experimental conditions.

Significance to Bio-medical Research and Program of the Institute: In the past nine years extensive study has been made on the subject of impedance changes of the brain tissues in animals of different physiological states. These changes are believed to be related to the change of the size of nerve cells and the change in extracellular space of the nervous tissue in the brain. However, the basic mechanism underlying this change in electrical measurements is not completely understood. The present investigation is designed for further understanding of this mechanism. The results of this study will serve as a test for the mathematical equation derived by one of the principal investigators (Cole) approximately 30 years ago.

Proposed Course of Project: This study will be extended from biophysical models to nervous tissues for further evaluation of the validity of the results reported by various investigators.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1526(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Epileptic Neurons and Their Recurrent Axon Collaterals

Previous Serial Number: SAME

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Anthony F. Bak

Cooperating Unit: M-LNP

Man Years:
Total: .2
Professional: .1
Other: .1

Project Description:

Objective: To study the electrical epileptic nerve cells in the cerebral cortex by means of intracellular microelectrodes. This study was also extended to investigate the impedance change, capacitance and time constant of the active neurons in the motor cortex.

Methods Employed:

a. Cats under ether and d-tuborcurarine or under pentobarbitone sodium are used.
b. A glass micropipette electrode is positioned in the interior of a nerve cell in the cerebral cortex.
c. Electrical current of various intensity and duration is applied through the micropipette, and the electrical activity of the cell is recorded.
d. Stimuli are also applied to the surface of the cortex adjacent to the microelectrode. Surface electrical activity is also recorded.

Major Findings:

a. Depolarization of the nerve cell membrane with a constant current of appropriate strength and duration causes the cell to discharge at high frequencies comparable to epileptic

firing. The termination of depolarization sets off a prolonged hyperpolarization. There is no afterdischarge of the cell. This is in contrast to the observation usually obtained in epileptic cells produced by other methods.

b. Repetitive depolarization with short duration, constant current pulses at frequencies of 20-50 per second, evokes repetitive discharges of the cell. The termination of the repetitive depolarization again gives rise to a prolonged hypolarization during which time small ripples of inhibitory postsynaptic potentials appear. Again, there is no afterdischarge.
c. In both instances (a and b) there is no change in activity recorded from the cortical surface 1 mm. from the cell under study.
d. Hyperpolarization of the cell abolishes the activity of the cell with absence of miniature excitatory postsynaptic potentials.
e. Anodal break responses may occur, depending upon the level of the resting membrane potential and the state of the discharge activity of the cell, before the application of hyperpolarizing current. In all instances there is no afterdischarge and no change in the surface cortical activity.
f. Repetitive stimulation applied to the cortical surface causes a gradual depolarization of the cell membrane with high frequency discharges. Epileptic afterdischarges appear on the surface associated with bursts of cell discharges.
g. The values of cell resistance, capacitance and time constant will be published.

Significance to Bio-medical Research and Program of the Institute: This study provides conclusive evidence that there is a negative feedback system; and the recipient neuron for the impinging impulses, which are generated by the neuron and travel along the recurrent axon collaterals, is the neuron itself. This is accounted for by the small ripples of potential and prolonged hyperpolarization following the termination of the repetitive depolarization pulses. The afterdischarges and epileptic activity require massive and synchronous synaptic activation which can occur only in a large population of cells. However, the size of a population capable of producing epilepsy in a clinical sense remains to be determined

Proposed Course of Project: The present investigation leads to the understanding of the mechanism of epilepsy and much more effort will be directed to this goal.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 SN/OC 1527(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Physiological Mechanism of Motor Function in the Cat

Previous Serial Number: Same and incorporating Serial No. NDS(I)-68 SN/OC 1524(c).

Principal Investigator: Choh-luh Li, M.D., Ph.D.

Other Investigators: Robert Ratcheson, M.D., T.C. Chen, Ph.D.

Cooperating Unit: N-OB

Man Years:
Total: .3
Professional: .2
Other: .1

Project Description:

Objective: To study the functional organization of the pyramidal and extrapyramidal pathway.

Methods Employed:

a. Multiple extracellular microelectrodes are placed in the motor cortex, nucleus ventralis lateralis of the thalamus caudate nuclei of the cerebellum.
b. Spontaneous extracellular spike action potentials are simultaneously recorded and stored in magnetic tapes.
c. The spike action potentials are converted into digital signals, and their relationship is to be computer analyzed.
d. Stimulations of various frequencies and strengths are applied to the dentate nucleus and cerebral peduncle. The responses of the cells in various structures are simultaneously recorded on tape and analyzed by the computer.
e. In some animals the motor cortex or one of the subcortical nuclear structures had been chronically destroyed and the relationship of the single cell discharges before and during stimulation is, likewise, analyzed.

Major Findings: The observation reveals that the dentate nucleus of

the cerebellum exerts strong influence on the activity of cells in the ipsilateral and contralateral red nucleus and of the somatosensory cortex. On some occasions spontaneous reciprocal activity of the cells in the motor cortex on the ipsilateral and contralateral side was seen. On other occasions the cell discharge in the red nucleus was facilitated by stimulation of the ipsilateral dentate and inhibited by the contralateral dentate, or vice versa. Finally, stimulation of the red nucleus on one side was found to evoke discharges from cells in the red nucleus on the opposite side. The results of other experiments will be further analyzed by the computer.

Significance to Bio-medical Research and Program of the Institute: Much has been learned about the functional connections at the cellular level between the cerebellar nuclei, basal ganglia and motor cortex (J. Neurosurg. Supp. Jan. 1966, Part II: 222-226 J. Exp. Neurol. 14: 319-327, March, 1966). The results of these studies were obtained with one or two extracellular microelectrodes simultaneously recording from one or two cells in the pyramidal and extrapyramidal pathways. With the present improved technology and knowledge of mathematics and computer, it is anticipated that with four or six microelectrodes recording simultaneously from different nuclear structures, a great deal more could be learned from the experiments presently designed.

Proposed Course of Project: This study will cover all the structures in the central nervous system related to motor function and will eventually be extended to study the functional relationship between the motor and sensory system.

Honors and Awards: None

Publications:

Bachman, D. and Li, Choh-luh: The effect of dentate stimulation on neuronal activity in the red nucleus. J. Exp. Neurol. 23: 58-66, 1969.

Li, Choh-luh, and Parker, L.O.: The effect of dentate stimulation on neuronal activity in the globus pallidus. J. Exp. Neurol. (In Press), 1969.

Serial No. NDS(I)-69 SN/OC 1658(c)
1. Surgical Neurology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Hemispheric Development and Specialization of Intellectual Functions.

Previous Serial Number: New Project

Principal Investigator: Paul Fedio, Ph.D.

Other Investigators: M. Baldwin, M.D., J. M. Van Buren, M.D., Ph.D., A.K. Ommaya, F.R.C.S., R. Minsky, Ed.D., S. Goodman, Ph.D., A.F. Mirsky, Ph.D.

Cooperating Units: Division of Research and Psychological Services, Montgomery County Schools, Maryland; Division of Psychiatry, Boston University.

Man Years
Total: .2
Professional: .2
Other: .0

Project Description

Objectives: 1) Compare effects of early and late brain damage on the development of intellectual abilities.

2) Examine cerebral competence in perception and memory as related to the acquisition of verbal skills.

3) Outline neural code for information storage and retrieval after thalamotomy or temporal lobe surgery.

Methods Employed:

1. Psychological evaluation of children and adults with similar cortical or subcortical involvement.

2. Tachistoscopic and dichotic perception in children with reading disorders.

3. Specialized testing of attention, short term and long term memory in adult neurosurgical patients.

Major Findings: Children, 6 to 14 years of age, with unilateral temporal lobe or centrencephalic epilepsy were presented visual and auditory tasks of verbal and nonverbal memory. Patients with left temporal involvement showed learning and memory deficits on verbal tasks while their performance on nonverbal tests was unaffected. The converse appeared to be true for children with right temporal epilepsy. In contrast to the two temporal groups, children with subcortical or centrencephalic epilepsy were without significant memory defect; instead, they earned lower scores on a test of sustained attention. The results are similar to those obtained in studies of adults with comparable cerebral dysfunction.

The findings suggest that cerebral differentiation of intellectual processes is established during childhood and is subject to selective impairment following early injury. The study also indicates that the presence of early injury in the left hemisphere may be compatible with continued, albeit less efficient mediation of verbal functions through the damaged (left) hemisphere. However, if an early lesion encroaches directly on the classical language zone in the left hemisphere, speech appears to be subserved by the right hemisphere. Thus, the location and size of lesion in the left hemisphere interacts with age (time of injury) in shaping the likelihood of right hemisphere dominance for speech.

Significance to Bio-medical Research and the Program of the Institute: The investigations advance understanding of the development and organization of structural-functional relationships in the human central nervous system. The information also turns attention to cerebral dysfunctioning which produces dysphasia, dyslexia and kindred language disorders.

Proposed Course of Project: The assortment of behavioral tasks employed in the study of epileptic children will be modified and presented to adult patients with lateralized cortical and subcortical lesions. A study of inter-hemispheric relations in perceptual style will be undertaken with dyslexic children.

Honors and Awards: None

Publications:

Fedio, P. and Mirsky, A.F.: Selective intellectual deficits in children with temporal lobe or centrencephalic epilepsy. Neuropsychologia (in press).

Serial No. NDS(I)-69 SN/OC 1727(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental Hydrocephalus

Previous Serial Number: New Project

Principal Investigator: Thomas H. Milhorat, M.D.

Other Investigators: Ronald G. Clark, Ph.D., Mary K. Hammock, M.D., Maitland Baldwin, M.D.

Cooperating Units: BS-LP

Man Years
Total: 2.5
Professional: 2.0
Other: 0.5

Project Description:

Objectives:

a. To develop an experimental model for obstructive hydrocephalus in the primate.
b. To study the gross and microscopic pathology subsequent to such developments.
c. To study fluid transport and other functional concomitants of the systems of the obstructive hydrocephalus.

Methods Employed: The Rhesus monkey was used for this study. In this animal a small bilateral suboccipital craniotomy was made through a posterior midline incision. Defects in bone and dura were made as small as possible to reduce the effects of surgical decompression. The foramen of Magendie was dilated with a blunt-nosed staphylorrhaphy near the cavity of the fourth ventricle. By this maneuver the orifice of the foramen was easily widened, and a No. 8 Foley catheter was then introduced into the fourth ventricle and advanced to the level of the caudal aqueduct. Once the catheter was in place, its balloon was inflated with saline so as to produce a mass of approximately 1-1.5 cc. volume. The catheter was then tied off with ligatures; thus, the cavity of the fourth ventricle was sealed and the exit to the caudal aqueduct blocked. All of this was accomplished under light Sernylan anesthesia with adequate aseptic and other precautions for the safety and welfare of the animal.

Major Findings: In the 230 monkeys in which this technique was applied, gross ventricular dilatation was apparent in a matter of hours. There were no failures in the surviving animals and ventricular enlargement was well advanced within 3 hours. Serial ventriculograms were performed in some animals, as were serial examinations of CSF, and the resultant gross pathology was carefully examined. After complete recovery from anesthesia, none of these animals were considered in the normally alert state of wakefulness. For the most part they sat quietly and assumed a stooped posture with their heads upon their chests as though sleeping. However, they remained self-sufficient with regard to feeding and other cage habits. In the early days after obstruction, truncal ataxia, dysmetria of the extremities and, at times, intention tremor developed. However, these signs were generally transient and became progressively less prominent after 48-72 hours. Cranial nerves were observed in some as was a decreased gag reflex with an impaired facility for swallowing solid foods. Vomiting was a rare finding.

The hydrocephalus developed as early as one hour, and in 3 hours specimens were shown to be quite advanced. Thereafter, ventricular size continued to increase, but more slowly. Actually, the size of the lateral ventricles within 3-6 hours after obstruction was at least 50% of that reached 2 weeks after obstruction. The early enlargement of the ventricular system was characterized by expansion which preserved the angulated ventricular contours for at least 12-14 hours. Thereafter, rounding of the ventricular angles and generalized remodelling of the cerebral architecture occurred. This was usually evident only after ventricular enlargement had become well established. There was a consistent sequence of enlargement characterized by initial expansion of the lateral ventricles, followed in turn by the third ventricle and then by the aqueduct of Sylvius. However, the enlargement of the third and lateral ventricles was not uniform, and within each ventricle certain areas became involved before others. The frontal horns were most prominently affected and most rapidly changed; the occipital horns, temporal horns and bodies of the lateral ventricles follow. The anterior and inferior aspects of the third ventricle were only slightly involved in the dilatation save for some thinning of the ventricular floor. The most prominent enlargement of the third ventricle was seen in the suprapineal recess. Simultaneously, the corpus callosum became slightly elevated in the midline and lost its normal ventral angulation. This probably resulted from expansion of the lateral and third ventricle with subsequent elevation of the ventricular roofs. At 3 hours there was enlargement of the lateral and third ventricles, which was advanced, and slight dilatation of the aqueduct of Sylvius. Ventricular size increased only slightly at 6 hours and the rate of enlargement, although continuing, was slowed markedly at 12 hours, as at 18 and 24. In the 24 hour specimen, the massa intermedia seemed to have disappeared. The septum pellucidum was thinned to the point of transparency. The third ventricle was capacious and seemed a single chamber running from the foramen of Monro downwards.

Significance to Bio-medical Research and Program of the Institute: This is the first successful experimental model of obstructive hydrocephalus in the primate. It has been consistently replicated in 230 monkeys, and, as a

subsequent basis for study of morphological and functional changes, it seems quite promising.

Proposed Course of Project: The experimental model will be used for a study of pressures, flow rates and active transport systems.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-60 SN/NA 702(c)
1. Surgical Neurology
2. Office of Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental Hypothermia

Previous Serial Number: SAME

Principal Investigator: Maitland Baldwin, M. D.

Other Investigators: Y. Kondo, M. D., J. Butterfield, M. D.

Cooperating Units: R-BEI

Man Years
Total: 3.0
Professional: 2.0
Other: 1.0

Project Description:

Objectives:

a. To study the effects of low temperature on the brain.
b. To study various related physiological parameters.
c. The development of topical and selective cooling techniques potentially available for clinical application.

Methods Employed: The methods employed are body surface cooling, topical cooling of the exposed brain, and/or spinal cord, intravascular perfusion, and combinations of these.

Major Findings:

a. A surface cooling method for brain hypothermia suitable for clinical application.

b. A combination of intravascular and head surface cooling suitable for clinical application.

c. Experimental application of the latter combination to exsanguinated dogs 10 minutes after clinical death providing for resuscitation in 18 out of 20 animals.

d. Intravascular hypothermia with extracorporeal perfusion in support of isolated head preparations.

e. Observations on the ischemic brain, an experimental preparation, in which the heads of monkeys are exchanged with reanastomosis of carotid, jugular, and vertebral circulation. The latter preparations demonstrated eye blink, pupillary reflex, salivation, movements of mouth and jaws, rising brain temperatures, and significant PO_2, CO_2 differences in carotid jugular circulation.

Significance to Bio-medical Research and Program of the Institute: These projects have provided techniques for application in the clinical operating room, as well as the basis for experimental endeavors related to treatment of exsanguination and the problems of the ischemic brain.

Proposed Course of Project: The prototype model for the ischemic brain will be developed, and supporting hypothermia and the surface intravascular cooling techniques refined for further clinical application.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-60 SN/CN 706(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Clinical, Biochemical and Genetical Studies of Mental Retardation, Progressive Cerebral Degeneration and Cerebral Palsy in Children.

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D., Michael Kappy, M.D.,Ph.D., and Dennis Cain, Ph.D.

Cooperating Units: None

Man Years

Total:	1.4
Professional:	1.0
Other:	0.4

Project Description:

Objectives: This is a large permanent program. Chronic neurological disorders affecting children constitute one of the major medical and social problems of our times. According to conservative estimates for this country, about four million people are permanently handicapped by mental retardation, cerebral palsy, epilepsy or progressive cerebral degeneration. Many of them have to be cared for to the end of their life by the joined services of the society and government. Until recently these conditions did not receive sufficient attention in the field of medical research. Our main objectives are:

1. To subdivide the large number of studied patients with mental retardation, cerebral palsy and epilepsy into distinct clinical categories using developmental and neurological examinations along with special laboratory procedures.

2. To establish types of cerebral lesion characteristics of each category whenever possible.

3. To apply basic biochemical and genetic methods to selected diseases or syndromes within each category with the aim of advancing the underlying pathogenesis and etiology.

4. To advance when possible the therapy and prevention of these chronic diseases.

Methods Employed:

1. Detailed general medical and neurological studies.

2. Developmental and psychological examinations.

3. Modified electroencephalographic studies.

4. Biochemical assays of protein, lipid and carbohydrate metabolism.

5. Genetic and cytogenetic studies.

6. Employment of special procedures such as pneumoencephalography and biochemical studies of brain biopsies whenever these are performed for diagnostic reasons.

Patient Material: 31 inpatients and 14 outpatients were studied under this project.

Clinical Project:

Major Findings:

1. The distribution of patients in regard to their diagnosis is as follows: 11 had Hunter-Hurler's or Morquio's syndrome, 9 had cerebral lipidosis or diffuse sclerosis, 8 had other progressive degeneration or genetic conditions (Lafora body disease, congenital retinal blindness) and the remainder had miscellaneous disorders including chromosomal aberrations.

2. The progress in understanding pathogenesis and assessing therapeutic trials in mucopolysaccharoidoses, especially Hunter-Hurler's syndrome, was handicapped by the lack of advanced methods which would permit us to assess objectively changes in the natural course of the disease. We were fortunate to have developed a technique which permits relatively easy determination of molecular weights of acid mucopolysaccharides and also their elution diagrams which are characteristic of different variants of the disease. They also permit us to follow the improvement or regression of this disease. The details of the method are described in the paper listed below. It was found that a linear relationship exists between the elution volume and the log of molecular weight of the respective mucopolysaccharide. The technique permitted demonstration that the accumulating in excess chondroitin sulfate B and heparitin sulfate in different variants of the disease are markedly degraded and of low molecular weight as compared to similar substances in normal controls.

3. Eight patients with Hunter-Hurler's syndrome were classified into three variants according to the composition of the acid mucopolysaccharides (AMPS)

excreted in their urine and the mode of inheritance of the trait. All these patients had AMPS inclusions in a proportion of their lymphocytes. The ratio of the lymphocytes with inclusions in the peripheral blood remained quite constant in individual patients over 1-2 years of follow-up. However, this ratio varied considerably in different patients; the predominantly heparitin excreters had generally higher ratio of inclusion containing lymphocytes than mixed excreters. The following tabulated data on the long-range persistence of the inclusions in lymphocytes from the peripheral blood gives detailed information on the ratios in different genetic variants of the disease.

In vitro studies showed that biological behavior of the lymphocytes with inclusions does not differ noticeably from the inclusion free lymphocytes in patients and in normal controls. Toluidin blue staining test of blood smears proved to be a simple and reliable diagnostic procedure for Hunter-Hurler's syndrome.

4. Our previous study on radiation injury to the developing human fetus demonstrated among others that microencephaly results from x-ray exposure during any time between one and six months gestation. Since the brain is undergoing rapid differentiation and growth during this long stretch of time, we had to assume that different types of lesions must be produced when radiation occurs during progressive stages of maturation. However, the nature of selective vulnerability to irradiation of different cerebral structures was unknown. Experimental study in irradiating developing mouse fetuses provided a source for some answers. Thus, the true or organ-type malformations (dysraphism, single ventricle brain) were encountered following irradiation on the 8th and 9th gestation days. Following irradiation between the 9th and 17th days of gestation the abnormalities consisted of foci or parenchymal degeneration, regenerative heterotopias and finally minor cortical imperfections. The least sensitive to irradiation during this stage were structures of paleopallium (prepyriform and enthorhinal areas of olfactory bulbs); the diencephalic structures were moderately sensitive, the archipallium (hippocampus) was considerably more sensitive and the neopallium exhibited the greatest sensitivity to irradiation of all brain subdivisions.

Significance to Bio-Medical Research and the Program of the Institute: The described findings under 1-4 constitute significant contribution to the research on metabolic and chronic neurological disorders in children.

Proposed Course of the Project: This field of study is one of the most important in medicine at the present time; therefore, this program is expected to continue for many years to come.

Honors and Awards:

1. Clinical Assistant Professor of Neurology, George Washington University.
2. Consultant, Children's Hospital of the District of Columbia.

Per Cent and Standard Error of Mean of Lymphocytes with AMPS Inclusions in Peripheral Blood During Follow-up Examinations

Patient (Age-Yrs.)	Urinary AMPS (Mg/24 Hrs.)	Per Cent of Lymphocytes with Inclusions				
		Initial	6 mos.	12 mos.	18 mos.	24 mos.
K.J. (5)*	141	13.2 ± 1.5	12.4 ± 1.4	--	14.6 ± 2.3	--
M.H. (10)*	225	14.0 ± 3.4	20.0 ± 1.2	13.1 ± 2.3	21.7 ± 2.0	20.0 ± 1.26
V.R. (5)*	181	8.5 ± 2.7	--	--	11.2 ± 1.8	--
R.J. (1)*	116	31.5 ± 1.4	30.5 ± 1.4	28.2 ± 1.4	26.0 ± 1.3	--
J.P. (6)^{X}	112	26.0	--	--	--	--
V.V. (8)**	114	42.2 ± 2.2	43.0 ± 1.5	43.0 ± 1.5	46.0 ± 2.1	42.0 ± 1.56
A.L. (8)**	51	27.4 ± 2.3	--	--	--	--
A.B. (6)**	134	36.0 ± 2.1	33.0 ± 1.4	32.0 ± 1.4	--	--

* Autosomal recessive mixed excreter
X X-linked mixed excreter
** Autosomal recessive heparitin excreter

Serial No. NDS(I)-60 SN/CN 706(c)

1. Constantopoulos, G., Dekaban, A.S., and Carroll, W.R.: Determination of the molecular weight distribution of acid mucopolysacciarides by Sephadex gel filtration. Anal. Biochem., In press.

2. Zelson, J. and Dekaban, A.S.: Biological behavior of lymphocytes in Hunter-Hurler's Disease. Arch. Neurol. 20: 358-361, 1969.

3. Dekaban, A.S.: Differential vulnerability to irradiation of various cerebral structures during prenatal development. Ninth Annual Hanford Biology Symposium on Radiation Biology of the Fetal and Juvenile Animals, In press.

Serial No. NDS(I)-60 SN/CN 707(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Study of Pathological Lesions of the Central Nervous System Occurring During Prenatal, Intranatal and Early Postnatal Life

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Dennis Cain, Ph.D., Jan Steusing, and John Van Buren, M.D.

Cooperating Units: None

Man Years

Total:	1.3
Professional:	0.8
Other:	0.5

Project Description:

Objectives: In the majority of patients the causation and the underlying pathology of mental deficiency and cerebral palsy are poorly understood. Detailed histological, histochemical and biochemical examination of the brains of such children are conducted. The main purpose is to correlate the encountered lesions with the clinical data. The accumulation of a larger body of data is expected to provide valuable information regarding pathogenesis and also clues for certain etiological factors. Limited, experimental approach in animals is used whenever indicated.

Methods Employed:

1. Detailed evaluation of the clinical and laboratory data of children who suffer from chronic neurological disorders.

2. Gross examination of brains after their demise.

3. Microscopical study of large histological sections stained by a variety of chromatic, myelin and silver methods as well as by special histochemical procedures.

4. Histochemical and chemical studies of brains and organs of patients who died of progressive cerebral degeneration and of inborn errors of metabolism affecting the nervous system.

5. Tissue culture of affected organs of patients with metabolic diseases with a view of studying cellular metabolism.

Material: Brains of patients who died with a diagnosis of cerebral palsy, epilepsy, mental deficiency or progressive cerebral degeneration. Brains of experimental animals.

Major Findings: Clinical-pathological correlations in certain neurological disorders are still scanty and there exists a need for clues to point the direction toward better oriented therapeutic and preventive measures.

1. Eight brains of patients with cerebral palsy, epilepsy or metabolic disorders affecting the CNS were processed using large histological sections and by histochemical methods. Three of these were particularly valuable since they represent one each: metachromatic leukodystrophy, Unverricht epilepsy and cerebral lipidosis. Some of the material was used in fresh state for biochemical studies which are still in progress. The histological and gross pathological examination of these eight brains increases our collection and will be used for comprehensive analysis when sufficient number of cases is prepared.

2. Variable amount of tissue from brain biopsies was obtained from six patients. Cerebral cortex and white matter were separated and they are currently being examined for lipid and protein composition.

3. In order to determine the time of greatest susceptibility to ionizing radiation of the developing fetus and to establish survival rates and type of cerebral abnormality detectable at birth, a large scale experimental study was carried out. A total of 152 female mice were irradiated on consecutive days of pregnancy between the 7th and 18th gestational day (g.d.). This resulted in 876 irradiated newborns. The mean number per litter after irradiation (200 R) on the 7th gestation day (g.d.) was 2.2, on the 8th g.d. 3.6 and on the 9th g.d. 4.6. The number of animals in the litter increased as the fetuses became older at the time of irradiation and after the 12th g.d. the litter size was between 7 and 8 which is close to the normal range for this strain of mice. Early resorption of selectively damaged fetuses was responsible for the small size of the litter when irradiation occurred during early stages of gestation. In many of these pregnant females either several placentas alone or placentas with disintegrated fetal tissues were observed.

Exposure to irradiation on the 7th and 8th g.d. led to death and absorption of the majority of the fetuses; however, those which survived were largely normal at birth and only a small proportion had organ-type brain abnormality. Irradiation on 9th g.d. produced an increased number of cerebral abnormalities which included dysraphism, hydrocephalus, microencephaly and arrhincephaly.

Following irradiation between 10 and 12 g.d. entirely different cerebral

lesions were found to occur; they included heterotopias, deformities of various structures, cellular deficiency and compensatory dilatation of the ventricles. From the 13th g.d. onward these lesions became smaller and less frequent, and after the 15th g.d. only mild architectonic imperfections were present. The heterotopias were most prominent in the subependymal region when irradiation occurred on the 10th and 11th g.d., in the white matter after exposure on the 12th g.d. and in the cerebral cortex following irradiation on the 13th and 14th g.d.

Details on types of cerebral abnormalities and their incidence in relation to the stage of gestation during irradiation are summarized in a tabulated form below.

DETAILED DISTRIBUTION OF TYPES OF ABNORMALITIES

Irradiated gest. days	No. abnor. brains from col. 3, Table 2	Malform. of early organogenesis	Heterotopic cell masses: rosettes, cords, clumps; damage & repair
7	2 (8%)	2 { 1 exencephaly 1 hydroceph.	--
8	9 (16%)	9 { 2 exencephaly 2 single ventr. 1 encephalocele 4 hydrocepha.	--
9	24 (37%)	17 { 3 encephaloceles 3 single ventr. 11 hydroceph. mild 2 mod. 4 mark. 5	7 few (dienc. & olf.)
10	141(98%)	11 { 3 hydroceph. mod. 8 HTV*	133 { small am't. 19 mod. am't. 31 many 83
11	162(100%)	3 HTV*	161 { small am't. 12 mod. am't. 38 many 111
12	67(100%)	--	67 { small am't. 42 mod. am't. 16 many 9
13	59(100%)	--	59 { small am't. 24 mod. am't. 29 many 6
14	56(98%)	--	55 { few 39 small am't. 14 mod. am't. 2
15	26(50%)	--	**
16	8(19%)	--	**

Irradiated gest. days	No. abnor. brains from col. 3, Table 2	Malform. of early organogenesis	Heterotopic cell masses: rosettes, cords, clumps; damage & repair
17	2 (5%)	--	**
18	†	--	--

* HTV - High situation of the third ventricle.
** Mild imperfection in neopallium.
† Rare necrotic cells.

4. This study provides us with very important information on pathogenesis of true congenital malformations and also destructive cerebral lesions as seen randomly in our patients and in the brains examined by us of the deceased patients.

Significance to Bio-Medical Research and the Program of the Institute: Knowledge of detailed pathology and pathogenesis of numerous conditions which underlie cerebral palsy, mental retardation and progressive cerebral degeneration is of basic importance for eventual therapeutic and preventive measures. Some of our studies in this field are applied type and some as the above quoted have basic implication of vulnerability and selective ability at regeneration of the developing brain. The disorders listed are clearly the major responsibility of our institute.

Proposed Course of the Project: This project has a wide scope and pertains to one of the major objectives of the Section on Child Neurology and also of NINDS; therefore, it is considered as our permanent project.

Honors and Awards:

None

Publications:

Dekaban, A.S.: Effects of x-radiation on mouse fetus during gestation: emphasis on the distribution of cerebral lesions. J. Nucl. Med. 10: 68-77, 1969.

Serial No. NDS(I)-60 SN/CN 708(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cytogenetical Study of Human Chromosomes Especially in Patients with Congenital Malformations and Mongoloids and Their Families

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: None

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: A proportion of patients with congenital malformations and mental retardation have chromosomal aberrations. The aberrations may occur sporadically, in which case they represent an accident of meiosis or division during early cleavage or the chromosomal aberrations may be present in many members of the family. In this last instance it is heritable according to the laws of Mendel.

Methods Employed:

1. Comprehensive clinical, laboratory and pedigree investigation of selected patients to establish diagnosis and extent of abnormality.

2. Tissue culture of lymphocytes from the peripheral blood and fibroblasts from skin. Bacto-phytohemagglutinin is added in order to stimulate lymphocyte proliferation. Six hours prior to the sacrificing the culture colchicine is added in a final concentration of 0.5×10^{-6}. Fibroblast cultures are similarly processed.

3. Through a number of steps the white blood cell suspension is centrifuged and washed, then exposed to hypotonic salt solution and fixed.

4. Concentrated suspension of cells is stained on the slide with orcein, Giemsa or Feulgen methods.

5. Under high power resolution in light and contrast phase microscope the chromosomes are counted, their morphology studied and karyotype figures made for final analysis.

6. In selected patients, DNA duplication sequence is followed in the chromosomes during the late phase of lymphocyte culture using tritium labeled thymidine.

Material: Patients with congenital malformations, mental retardation and mongolism, as well as their families are the subject of this study. An experimental approach on human tissue cultures is also used.

Major Findings:

1. Karyotypes were prepared from cultured leukocytes and fibroblasts of twelve patients and seven members of their families suspected of having chromosomal abnormalities. Of these 19 subjects seven had chromosomal aberrations; these included Turner's syndrome with retardation in one, D/D translocation in one, mongolism in four and an interesting anomaly in G group of chromosomes in one, to be described below in detail.

2. A retarded girl, age 4 years was studied in great detail. Her physical appearance and general health were normal. However, she had 47 chromosomes, the additional one being atypical and unlike the remainder of the chromosomes. In length it was only 2/3 of the shortest chromosomes of group G and its centromere was situated in the submetacentric position. Measurements, DNA replication studies, palmar dermatoglyphics and radiological analysis of the skeleton were consistent with partial mongolism and a probable deletion-translocation involving one of the chromosomes of group G. We will continue to follow-up this patient since this type of phenotype and genotype will eventually render itself to chromosome mapping in man.

Significance to Bio-Medical Research and the Program of the Institute: Study of abnormal chromosomes in clinical syndromes provides the only mean whereby human chromosomes could be mapped; the importance of this cannot be overemphasized. The etiology and pathogenesis of a vast number of conditions associated with congenital malformation and mental deficiency are largely unknown. Demonstration of chromosomal aberration in some of these patients or their parents is a great step forward in our understanding of these conditions and it may suggest certain preventive measures. Also, it is a tool for future chromosome mapping in man.

Proposed Course of the Project: Terminated. Because of smallness of our staff and space limitation we are forced to discontinue this project. During the past four years we have published six papers which have contributed significant new data to various aspects of human chromosomes, including sensitivity to radiation.

Honors and Awards: None

Publications: None (See above -- "Proposed Course of the Project")

Serial No. NDS(I)-63 SN/CN 1026(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Clinical and Biochemical Studies on Epilepsy

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: Michael Kappy, M.D., Ph.D., Vicky Patton, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: Idiopathic epilepsy is a clinical syndrome of unknown etiologies. In the first instance it is important to accumulate a sufficient number of patients for the purpose of subdividing them into different categories depending on certain clinical and laboratory findings. This will permit better directed therapeutic application of certain regimens and drugs under investigation. The patient's response or its lack can provide us with some information on the pathogenesis of the condition.

Methods Employed:
1. Necessary clinical examinations and tests.
2. Routine and special electroencephalographic studies.
3. Pneumoencephalography and arteriography when indicated.
4. Urinary and plasma amino acids.
5. Assays of carbohydrate metabolism.
6. Plasma lipid studies.
7. Endocrinological assays when indicated.
8. Application of special therapeutic procedures.
9. Special studies on high fat diet.

Patient Material: 18 inpatients and 7 outpatients, most of them suffering from "idiopathic" epilepsy.

Major Findings:

1. Eighteen children suffering from frequent epileptic attacks (generally

ranging between 10-100+/day) were investigated in detail and treated during the past year as inpatients. Eleven of these patients were classified as having idiopathic epilepsy and seven as having organic basis of cerebral seizures. Eight patients of the idiopathic epilepsy variety were established on high fat diet regimen and all of them showed better control of seizures than it was possible to accomplish with any combination of drugs. These patients increased our series of children treated with this type of regimen and also provided an opportunity for special studies of acid base prior to and while on the diet. The results will be evaluated when we reach sufficient number of patients for statistical analysis.

2. Out of seven patients with organic type of epilepsy, five had generalized conditions (tuberous sclerosis, encephalitis, hypoglycemia) and two had unusual focal lesions of malformation type. These last two patients will be described in some detail now.

3. Two infant siblings, a male and a female, were investigated as inpatients about one year apart. The presenting symptoms consisted of retardation, seizures and blindness dating since birth. In addition, one of them had encephalocele and its repair provided opportunity for a small brain biopsy. The postmortem examination was available in the second sibling. The abnormalities detected included deficient differentiation of the neural elements and of photoreceptors. Analysis of all findings permitted determination of the stage when embryonal differentiation was disturbed. Since we were dealing with a similar disorder in two siblings, this poses an interesting question of a possible genetic derangement of development of the human fetus.

Significance to Bio-Medical Research and the Program of the Institute: Epilepsy is a frequent disorder in all age groups and especially during childhood. Our understanding of the pathogenesis of seizures and their control is still meager. Many young children become severely and permanently retarded as a result of frequent seizures. Biochemical approach to epilepsy is likely to provide us with better understanding of this syndrome complex and it may help us in the therapy of this distressing disorder. The investigation enlarges the overall scope of the study on epilepsy which is a major theme of the Surgical Neurology Branch, NINDS.

Proposed Course of the Project: The project will continue for years to come.

Honors and Awards: None

Publications:

Dekaban, A.S.: Familial occurrence of congenital retinal blindness and developmental renal lesions. J. Hum. Genet., in press.

Serial No. NDS(I)-68 SN/CN 1523(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Lipid and Polysaccharide Composition of the White and Grey Matter of the Brain

Previous Serial Number: Same

Principal Investigator: Anatole S. Dekaban, M.D., Ph.D.

Other Investigators: George Constantopoulos, Ph.D. and Victoria Patton, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: Many of our patients with progressive cerebral degenerations have either excessive storage of a material in the nervous system (lipid, mucopolysaccharide, glycogen) or loss of structural material (diffuse sclerosis, subacute sclerosing encephalitis, axonal degeneration). Initially we plan to use experimental animals to establish biochemical techniques for analysis of partitioned lipids and mucopolysaccharides in subcellular fractions. Human material, both normal and abnormal, will be utilized principally as it becomes available.

Methods Employed:

1. Rapid removal of brain from deceased patients with progressive cerebral degeneration or those who are meant to be used as unaffected controls. Portions of the brain tissue are dissected and stored at -125°F for chemical studies.

2. Using recently available technique, separation of neurons from glial elements and nerve fibers in the fresh tissue will be carried out.

3. Selected blocks of brain tissue are taken and fixed in appropriate solutions for histological and histochemical studies.

4. Brain biopsies from patients with progressive cerebral degeneration are similarly processed.

5. A modified chloroform-methanol technique of lipid extraction is used, followed by separation and identification of components by means of various columns and TLC.

6. Extraction of acid mucopolysaccharides from brain and other organs using various solvents as well as digestion with papain and pronase.

7. Isolation of mucopolysaccharides by precipitation with cetylpyridinum chloride or using Ecteola column. This is followed by analytical identification of specific types of mucopolysaccharides and inclusive determination of molecular weights.

8. Establishment of fibroblast tissue cultures from respective patients for study of turnover of individual acid mucopolysaccharides using radioactive labels.

Major Findings:

1. Our data on the content and composition of acid mucopolysaccharides in the brain and other organs in patients with Hunter-Hurler's syndrome is in a prepublication phase and cannot be listed here in detail. The autosomal recessive mixed excreters contain in liver 80-100 times greater amount of chondroitin sulfate B and heparitin sulfate than unaffected controls. The content of these compounds in spleen is about 20-40 times higher in patients and in the skin the content is 15-20 times higher than in normal controls. The various acid mucopolysaccharides are differently distributed in the grey and white matter of the brain.

2. Modification of the total content and composition of various mucopolysaccharides in the fibroblasts from patients with different variants of the Hunter-Hurler's syndrome is in the process of investigation. Preliminary findings indicate that corticosteroids influence considerably the production and turnover of excessively stored mucopolysaccharides while vitamin A has little effect.

Material:

1. Human cerebral tissue obtained by biopsy for diagnostic purposes or at autopsy.

2. Tissue cultures obtained from the affected patients.

3. Experimental animals, predominantly rats.

Significance to Bio-Medical Research and the Program of the Institute:
The composition and turnover of lipids and mucopolysaccharides in the brain in health and in various cerebral degenerations require concentrated effort. Our knowledge of the etiology and pathogenesis of those conditions is very scanty. Clinical procedures leading to modification of excretion or disposition of certain metabolites have to precede therapeutic approaches. This is clearly one of the important objectives of our Institute.

Proposed Course of the Project: This project has just begun and it is xpected to continue for several years to come.

.. rs and Awards: None

ublications:

1. Dekaban, A.S. and Zelson, J.: Studies in the Hunter-Hurler's Syndrome. Trans. Amer. Neurol. Ass. 93: 75-78, 1968.

2. Constantopoulos, G.: Hunter-Hurler's Syndrome. Gel filtration and dialysis of urinary acid mucopolysaccharides. Nature, 220: 583-584, 1968.

Serial No. NDS(I)-69 SN/CN 1655(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of the Composition and Biosynthesis of Cerebral Proteins in Experimental Animals and in Man

Previous Serial Number: New Project

Principal Investigator: Dennis Cain, Ph.D.

Other Investigators: Michael Kappy, M.D., Ph.D. and Anatole Dekaban, M.D.,Ph.D.

Cooperating Units: None

Man Years

Total:	1.3
Professional:	1.3
Other:	0.0

Project Description:

Objectives: Brain proteins differ greatly from those of other tissues especially in the elevated levels of the acidic proteins and in the very rapid rate of synthesis and degradation -- a process without a functional correlate.

An understanding of the role of these specialized aspects of protein metabolism in normal nervous tissue requires the determination of the identity, composition and relative rates of synthesis and degradation, especially of the acidic proteins, as well as their localization within various cell types and subcellular organelles.

This information will allow an examination of the role of protein metabolism in certain neurological diseases.

Methods Employed:

Working initially with the guinea pig, various protein fractions are extracted and purified by salt fractionation, isoelectric precipitation, column chromatography, etc. These proteins are subsequently analyzed by electrophoresis in a soluble acrylamide gel system. The specific purified protein fractions are also characterized by amino acid analysis, sedimentation, and immunological procedures.

In turnover studies, the brain proteins are labelled in vivo by intraventricular injections or in vitro (tissue slices) with ^{14}C- or ^{3}H-leucine. Specific radioactivities of the individual protein fractions are determined by measurement of both protein and radioactivity after separation in the soluble acrylamide gel system which was devised for this work.

Several techniques will be tested for use in the separation of subcellular as well as whole cell units for the localization of the individual acidic proteins.

Material: Brains and other organs (for purpose of control) of various animal species will be used routinely. In addition, samples of brain obtained during surgery and at autopsy will be utilized.

Major Findings:

1. Examination of concentrated brain extracts directly or of isolated protein fractions on the soluble acrylamide gel electrophoresis system shows six major and 4-6 minor soluble proteins in the brain which are more acidic than albumin. In vivo turnover studies of five of these fractions demonstrated a rapid rate of synthesis and degradation not unlike that of the total soluble fraction. However, no evidence of selective very rapid synthesis and degradation, as was reported by Hyden for the protein S-100, was observed in these fractions.

2. In preparation for study of postmortem human material, a preliminary study of the postmortem changes in the brain proteins of the guinea pig was conducted. It was found that a significant fall in extractable brain proteins was observed during the first 30 minutes after death, but after this there was little change through 12 hours when the brain in situ was kept at room temperature.

Assays of amino acids, free amino nitrogen and hemoglobin in the extract indicate that neither blood shifts nor enzymatic degradation of the protein account for the changes observed. Analysis of those extracts on gel electrophoresis shows a progressive disappearance of the more slowly migrating protein fractions. Measurements of pH changes in vitro experiments suggest that the decrease in extractable proteins may be due to denaturation resulting from the postmortem increase of acidity.

3. Comparison of extracts from various organs indicated that one of the proteins identified as FS which migrates more rapidly than albumin was present only in brain extracts. This protein has been purified and further studies are in progress.

Significance to Bio-Medical Research and the Program of the Institute: There is some suggestive and still indirect evidence that specific cerebral proteins may be involved in the processes of memory and intellectual functions. There are large numbers of subjects with undifferentiated type of mental retardation who defy all investigative approaches in clarifying the

underlying abnormality. Because of several technical difficulties the brain proteins have not been thus far explored in these and many types of other patients. The availability to us of surgical and postmortem tissue of a number of hitherto obscure neurological diseases provides us with unique opportunity to study cerebral proteins in these patients. Greater understanding of these conditions is one of the research responsibilities of our Institute.

Proposed Course of the Project: This is a new project. As evident from the description, it will be a major, long-standing project of our Section.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 SN/CN 1656(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of the Acid Mucopolysaccharides in the CSF of Patients with Certain Neurological Diseases

Previous Serial Number: New Project

Principal Investigator: George Constantopoulos, Ph.D.

Other Investigators: Anatole S. Dekaban, M.D., Ph.D.

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.4
Other:	0.0

Project Description:

Objectives: Certain diseases such as Hunter-Hurler's syndrome, Morquio's disease and mastocytosis are associated with excessive storage of AMPS in liver, spleen, brain and other organs. Also, these patients excrete large amounts of various AMPS in their urine. Since the brain is severely affected in a majority of these patients it is important to know if AMPS accumulate in abnormal amounts in their cerebrospinal fluid.

Methods Employed:

1. Accumulation of large samples of cerebrospinal fluid from the respective patients and from unaffected controls.

2. Selection and modification of the best suited method for isolation of AMPS from the CSF. Among those to be tried are the method of precipitation of AMPS with cetylpyridinium chloride and separation on the Ecteola chloride column.

3. Determination of the hexuronic acid content in the purified AMPS.

4. Identification of individual AMPS by electrophoresis.

5. Determination of the distribution of molecular weights in the purified AMPS.

Major Findings:

1. The initial findings indicate that it is possible to determine quantitatively the AMPS in the CSF from normal subjects and affected patients.

2. Contrary to findings by other investigators (Friman 1967) our study indicates that patients with Hunter-Hurler's syndrome have increased content of AMPS as compared to the normal controls.

3. AMPS identified in the CSF from the patients are chondroitin sulfate B and heparitin sulfate with only traces of chondroitin sulfate A and C.

4. The determination of the distribution of molecular weights in the isolated AMPS and from the CSF is currently under study.

Significance to Bio-Medical Research and the Program of the Institute: Central nervous system is frequently affected in generalized metabolic disorders of genetic origin. Demonstration of abnormal metabolites or excessive amount of normal metabolites in the CSF of these patients is important for two reasons:

a. It provides an opportunity to study various aspects of pathogenesis of cerebral involvement in particular metabolic disorders.

b. It allows exploration of brain barrier for compounds in question.

Proposed Course of the Project: This is a new project. It will be carried out to its completion as outlined above.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 SN/CN 1657(c)
1. Surgical Neurology Branch
2. Section on Child Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Amino Acids Composition of Acid Mucopolysaccharides Isolated from Hunter-Hurler's Patients and Normal Controls

Previous Serial Number: New Project

Principal Investigator: Victoria Patton, Ph.D.

Other Investigators: George Constantopoulos, Ph.D., Anatole, S. Dekaban, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.4
Other:	0.0

Project Description:

Objectives: Acid mucopolysaccharides (AMPS) are compounds which are present in many organs and tissues and are indispensable for a variety of physiological processes such as blood coagulation, electrolytes, homeostasis, and others. The moiety of AMPS is bound to a peptide. In certain diseases such as Hunter-Hurler's and Morquio's syndrome, mastocytosis and others, AMPS accumulate excessively in spleen, liver, brain and skin, leading to severe damage of these organs and eventually causing early death. The reason for this excessive storage is unknown. Recently, we have demonstrated [see project NDS(I)-60 SN/CN 706(c)] that the molecular weights of the AMPS in these diseases are considerably lower than in normal controls. Here, our first objective is to establish quantitative and qualitative composition of amino acids in the peptide portion of the AMPS molecule. The second objective is to determine the particular amino acid or amino acids which link the peptide to the AMPS moiety.

Methods Employed:

1. Accumulation of many 24 hour urine specimens from the patients and normal controls under specified conditions.

2. Accumulation of samples from organs and tissue cultures from the patients by means of biopsy or at autopsy.

3. Isolation of AMPS by precipitation and extraction procedures (Cetyl-pyridinum chloride), followed in certain instances by digestion (papain) and re-extraction.

4. Isolation of AMPS on Ecteola column. This is a very mild procedure and least detrimental to the intactness of the molecules as they occur in life.

5. Purification of the isolated AMPS (Lloyd reagent).

6. The purified AMPS are acid hydrolyzed under vacuum and the free amino acids liberated are determined on a Technicon amino acid analyzer.

7. Alkaline treatment prior to acid hydrolysis is employed to determine the nature of the amino acid linking the peptide to the AMPS moiety.

8. The aminosugar of the AMPS is determined quantitatively and qualitatively following mild acid hydrolysis.

Major Findings: This is a new project. The initial findings indicate that:

1. The amino acid composition of the peptide bonded to AMPS moiety is strikingly similar in all three variants of Hunter-Hurler's syndrome (autosomal recessive mixed excreters, X-linked mixed excreters and heparitin excreters).

2. The predominant amino acids include serine, glycine and aspartic acid.

3. The amounts of these three amino acids relative to the hexosamine in AMPS of these patients are also very similar thus indicating that both chondroitin sulfate B and heparitin sulfate are associated with similar peptides.

Significance to Bio-Medical Research and the Program of the Institute: It is not known whether Hunter-Hurler's syndrome and other AMPS storage diseases are associated with the production of abnormal AMPS which cannot be degraded or if there is a defect in the degradation of a normal AMPS. Study of the protein moiety of AMPS obtained from patients and normal controls can provide important information to these questions.

Proposed Course of the Project: This is a new project. It is planned within the next 12-18 months to obtain the desired information and conclude the project.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-57 SN/CP 401(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Psychological Evaluation of Temporal Lobe Operations

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: M. Baldwin, M.D., A. Ommaya, F.R.C.S. and
J. Van Buren, M.D., Ph.D.

Cooperating Units: None

Man Years
Total: 0.7
Professional: 0.7
Other: 0.0

Project Description:

Objectives: To study neurosurgical patients with temporal lobe disorders with respect to intellectual abilities, visual and auditory perception, linguistic functions and other "personality" features.

Methods Employed: Intelligence, personality and other specialized verbal tests; tests of visual perception. A test of "stereotypy"--the production of random movement of a stick--uses a tape punch to record a 5-minute session three times a week.

Major Findings: By using Horst's new method, common and general factor score estimates were calculated for 42 patients in order to clarify some relationships previously reported between test scores and extent of neuropathology. Factor loadings were determined from test intercorrelations obtained from 146 intelligent patients who had been tested with Differential Aptitude Tests, Wechsler-Bellevue, Atwell & Wells's Vocabulary and Mooney's Closure Faces. Extent of surgery, or neuropathology, on the left was related to scores on a verbal factor and on the right to scores on a closure factor. The relation to scores on a general factor of "abstract reasoning" was independent of side of surgery.

Some years ago Dr. Davie provided a copy of his data on PEGs from patients with and without a massa intermedia (material was subsequently published). A recent analysis of some of these patients' test scores showed

that those with a massa intermedia had lower nonverbal scores on the Wechsler-Bellevue IQ test than those without it. The probable shrinkage of the brain with age, as shown by the area of the massa intermedia, also appeared to affect these scores. A careful check of the identity of each patient with Dr. Davie is in progress in order to eliminate some errors.

Significance to Bio-medical Research and the Program of the Institute: With careful selection of patients, relations between psychological test scores and extent of cerebral lesion can be established selectively for different loci and for each sex, and also independent of loci; these relationships have implications for physiological concepts of "higher mental" processes.

Proposed Course of Project: Further development of computer programs to analyze psychometric test results, publication of findings.

Honors and Awards: None

Publications:

Lansdell, H.: The use of factor scores from the Wechsler-Bellevue Scale of Intelligence in assessing patients with temporal lobe removals. Cortex 4: 257-268, 1968.

Serial No. NDS(I)-63 SN/CP 1032(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Psychological Effects of Subcortical Lesions Used for Relief from Abnormal Movements.

Previous Serial Number: SAME

Principal Investigator: Herbert Lansdell, Ph.D.

Other Investigators: J. Van Buren, M.D., Ph.D., and A. Ommaya, F.R.C.S.

Cooperating Units: None

Man Years
Total: 0.1
Professional: 0.1
Other: 0.0

Project Description:

Objective: In comparison with the effects of other forms of neurosurgery, to investigate the changes resulting from subcortical lesions with the aim of delineating subcortical from cortical factors in brain function.

Methods Employed: A battery of tests [the same as those used in Project Serial No. 401(c)] has been used on patients before, two weeks after, and again a year or more after operation.

Major Finding: A preliminary analysis of our word association test indicates, somewhat contrary to expectation, that the subcortical operations are followed by a side difference while the temporal operations are not; and the sex of the patients was relevant. More specifically, after left thalamotomy male patients made more errors in choosing the most popular association for the words in the test, whereas other surgical patients have not. This result is to be investigated further by a factor analysis of the test scores, along with other scores on standard tests (in a large group of patients), to understand the pertinent factor involved; complications involving the age factor in these patients presumably are present.

Significance to Bio-medical Research and the Program of the Institute: Except possibly for Porteus Mazes, no psychometric test has yet shown clear asymmetrical hemispheric contributions only at the subcortical level; if this

preliminary finding proves, upon careful analysis, to demonstrate such a phenomenon, it will provide a clue about the subcortical contributions to verbal cognitive processes.

Proposed Course of Project: Further analysis of the psychometric data.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 SN/CP 1033(c)
1. Surgical Neurology Branch
2. Section on Clinical Psychology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Phonemic Aspects of Dysnomia

Previous Serial Number: SAME

Principal Investigators: Herbert Lansdell, Ph.D. and Paul Fedio, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objective: To investigate the phonemic aspects of induced dysphasia and normal naming errors.

Methods Employed: Filmstrips and a projector are used. The filmstrips contain three orders of pictures; the names of the pictured objects have a wide range of phonemes in the initial and final positions. The nouns have a high frequency in the English language. The projector has an attached miniature screen; there is automatic control of exposure time from 30 to less than 0.5 sec/frame.

Major Findings: Data solely relevant in this project are being accumulated with its use during carotid Amytal tests. To date, there appears to be no discernible confirmation of the previous result regarding the influence of the initial and final phonemes on naming errors; the nature of the word substitutions unfortunately cannot be investigated with the present small number of observations.

A finding related to this area may be noted here. Eighteen right-hemisphere speech cases were investigated with regard to their early neurological history and the verbal factor on the Wechsler-Bellevue Intelligence Scale. These verbal scores appeared to be inversely related to age at initial symptom; the scores on nonverbal factors were positively related to age at onset.

Significance to Bio-medical Research and the Program of the Institute: Future different types of data may continue to extend our knowledge of the phonemic aspect of speech and delineate this clue about the physiological mechanisms of speech and verbal activity.

Proposed Course of Project: The phonemic aspect of results will be analyzed when sufficient data are obtained.

Honors and Awards: None

Publications:

Lansdell, H.: Verbal and nonverbal factors in right-hemisphere speech: relation to early neurological history. J. Comp. Physiol. Psychol., (in press).

HOW TO USE
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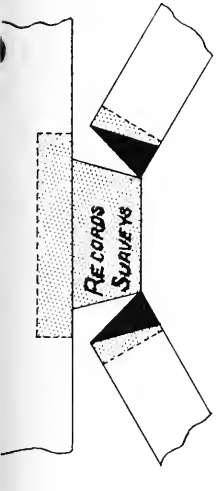

ANNUAL REPORT
July 1, 1968 through June 30, 1969
Branch of Ophthalmology, Intramural Research
National Institute of Neurological Diseases and Stroke

Ludwig von Sallmann, M.D., Chief

An important and most gratifying event of the past year, signaling a growth of the Ophthalmology Branch, was the addition, on September 8, 1968, of the Section on Experimental Embryology. Though only eight months have passed since this date the Section has taken part in the weekly NINDS conferences and in the monthly laboratory conferences of the Branch and has contributed an unusual number of manuscripts which reflects the many-sided and interesting research efforts. The trustees of the Association for Research in Ophthalmology bestowed on Dr. Alfred Coulombre, the Head of this Section, the Jonas Friedenwald Memorial Award, the highest sign of recognition of the society for a distinguished scientist. There is no doubt that ophthalmology and the Branch will continue to profit from this Section's work on basic problems of developmental interactions of the eye tissues and the studies of intrinsic and external factors which control such interactions.

There were no other changes in the ranks of the professional staff in view of current restrictions on filling vacant positions, except for clinical and research associates on two year appointment.

The number of patients admitted to the nursing unit and the inpatient days varied little from the figures reported last year. Slightly fewer patients were hospitalized in the Branch this year but the number of inpatient days exceeded those recorded last year. This discrepancy is explained by the longer hospitalization required for more elaborate immunologic studies on patients with uveitis. Application of new diagnostic procedures such as fluorescein angiography, ultrasound evaluation, and the great number of recently developed laboratory tests led to a 20% increase of outpatient examinations (2775) although the number of new outpatients fell about 12%. Requests for examination of patients from other units or Institutes reached 1684, a new peak. Despite the use of time consuming new tests in examination, the competence and dedication of the four clinical associates made it possible to carry this load most satisfactorily.

Laboratory Activities: Studies on morphology and physiology of the retina were the center of investigative attempts in five of the six sections. This concentrated laboratory effort supplemented clinical research on degenerative and vascular diseases of the retina.

The section on Ophthalmology Physiology has continued investigation of the functional organization of the monkey retina at the cellular level. In earlier studies each of the three cone mechanisms of primate vision were identified by recording from single retinal ganglion cells. There now appears to be at least 14 varieties of retinal ganglion cells differing in their receptive field organization. These include seven on-center and seven off-center groups with phasically and tonically responding cells represented in each group. Phasic cells are encountered most frequently away from the fovea and receive both excitatory and inhibitory signals from red and green sensitive cones and from rods. Tonic cells are common near the fovea and receive signals from only a single cone mechanism in the center of the receptive field and antagonistic signals from another mechanism in the periphery of the receptive field. It has been demonstrated that the tonic cells conduct impulses more slowly than phasic cells and therefore must be of smaller size. This observation lends further to support the concept that tonic cells belong to the midget cell system described by Polyak.

A normal ERG and optic nerve responses have been recorded from isolated perfused cat eyes maintained for up to 10 hours after removal from the animal. That visual pigment regeneration is maintained in these preparations is evident from the observation that the eye undergoes normal dark adaptation following exposure to very strong bleaching light.

An attempt is being made to record intracellularly from single vertebrate photoreceptors of Necturus maculosus. So far, the membrane potentials of these cells are positive internally and have not shown responses to light.

In an important study of the Section on Cell Biology, the fine structure of synaptic contacts of visual cells was investigated in the turtle retina. This tissue appears suitable to combine electronmicroscopic examinations with microelectrode recordings as the neurons are of relatively large size. It was observed that junctional areas at the basal surface of cone pedicles are similar to the septate junctions of the invertebrate eye. The

latter junctions are thought to represent points of electrical coupling between cells.

In the same section the search for the origin of electrical responses of photoreceptor cells in the leech was completed by appropriate resistance measurements. It was concluded that the channel connecting the vacuole to the outside fluid is an effective pathway for the receptor current. The resistance of these channels is similar to what would be expected if they were filled with Ringer's solution alone in spite of the bridging of the channel lumen by junctional structures.

The Section on Ophthalmic Chemistry has engaged in extensive studies of the chemistry of rhodopsin. Retinal outer segment membranes have been sonically disrupted to form particles with diameters of 50 Å to 200 Å. Circular dichroism measurements showed that these particles had the same extent of helical structure as purified rhodopsin, but unlike rhodopsin, did not undergo a loss of helical structure upon bleaching. These particles were found to possess a Na^+-K^+ dependent ATPase activity like that of the outer segments. Highly purified rhodopsin has been delipidated and chemically cleaved with cyanogen bromide. The resultant peptides were isolated by a peptide mapping procedure. The amino acid composition of one of these peptides which has associated carbohydrate, has been obtained. Ultracentrifugal studies of molecular weight have been done on native rhodopsin in buffered detergent solutions, on succinylated rhodopsin in aqueous buffers, and on delipidated rhodopsin in organic solvents. Equations have been derived to permit the calculation of the molecular weight of the rhodopsin in the rhodopsin-detergent complex. A molecular weight of 35,000 was obtained here, and an identical value was obtained for the delipidated rhodopsin. The succinyl rhodopsin was found to have a molecular weight of twice this value. In addition to establishing the molecular weight of rhodopsin, these studies also suggest that the rhodopsin molecule has an extensive, strongly hydrophobic region which is responsible for the dimer formation of the succinyl derivative and may be of considerable significance in terms of the role of the rhodopsin molecule in the structure of the outer segment membrane.

The water-soluble proteins of the lenses of embryonic and newly hatched chicks have been used for studies of model systems pertaining to transparency and opacity of gels. These proteins have been studied by a variety of fractionating and analytical techniques. In particular, gel electrophoresis has been used to

assist in the identification and semi-quantitation of fractions obtained by precipitation at various pH's. The molecular weights of the two lightest components have been found to be 113,000 and 270,000, each approximately double the molecular weights reported for β-crystallin and δ-crystallin, respectively, in other species. These studies are intended to relate the time of appearance, the relative quantities, and the physical and chemical properties of the water-soluble lens proteins to the development of the embryonic lens.

The physical and chemical properties of soluble vitreous humor proteins and glycoproteins have been studied in a similar manner. A major component obtained chromatographically has been further purified by a procedure used for serum orsomucoid. Ultracentrifugal analysis gave a molecular weight of 55,000 for the major component (96%) and a molecular weight of 295,000 for the minor component (4%). This study is a part of a continuing investigation into the relationship of the physical and chemical properties of the components of the vitreous humor to its structure and function.

Haemoproteins obtained from retinal pigment epithelial microsomes have been studied to attempt to elucidate their role in retinal metabolism. Model experiments with polyamino acids suggested that histidine was a probable binding site for the haem group in the P420 isolated from the pigment epithelium. This was supported by studies on the effects of succinylation and diazotization on the spectrum of this haemoprotein. Other studies indicated that tyrosine might also be involved in the hypochromic behavior of P420. The structure of the haem binding site of this haemoprotein may be of significance in characterizing its chromophore structure and further elucidating its various roles in retinal metabolism.

In the Section on Ophthalmic Metabolism, studies have been continued on the detailed mechanism of glycoprotein synthesis in the bovine retina. An enzyme has been detected which mediates the addition of the sugar, mannose, to newly-formed protein. This brings to three the number of such sugar transfer enzymes identified in the retina and accounts for the synthesis of three of the four sugar residues most commonly found in glycoproteins. All three enzymes function optimally at low levels of the respective sugar nucleotide donors and therefore represent an efficient synthetic mechanism capable of responding rapidly to a need for increased glycoprotein synthesis. Column chromatography of the radioactive products of two of these transfer

reactions indicate an apparent identity of the mannose labeled product with one of the products of sialic acid transfer. Thus there is evidence that these enzymes may be involved in the biosynthesis of a common membrane component.

The studies on the biosynthesis of rhodopsin in vitro have progressed using whole bovine retina. Radioactive amino acids are taken up by the retina and appear in rhodopsin, the amount increasing with time. The rhodopsin is reduced by sodium borohydride while outer segments are still suspended in buffer with no detergent present. Chemical degradation is thus avoided. After reduction, the stable N-retinyl opsin is extracted with detergent and purified by column chromatography and acrylamide gel electrophoresis. In addition to these improved methods of isolation and purification, it has been found possible to recover N-retinyl opsin without detectable change after precipitation with cold alcohol-ether. With these methods it has now become possible to study the environmental factors that control the biosynthesis of rhodopsin.

The Section on Experimental Embryology seeks to identify and to characterize the factors which govern the orderly emergence during development of structural organization in the eye and in the central visual system. Since the eye is a living optical instrument the size, shape and orientation of its component parts must be held within narrow geometric tolerances in order for it to function properly. The central visual system requires for its normal function the establishment and maintenance of intricate and precisely interconnected neuronal circuits.

This year the findings of the Section relate to the cornea, the lens, the retino-tectal connections and the centripetal connections which reach the neural retina from the isthmo-optic nucleus. The work utilized the easily accessible embryo of the domestic fowl.

Conclusive evidence was obtained that the entire collagenous structure of the corneal stroma is laid down in miniature beneath the anterior epithelium of the cornea before the stroma fibroblasts have invaded. In addition it has been established that successive lamellae are deposited seriatim beneath the anterior corneal epithelium. These results have significant consequences. It suggests that the orderly arrangement of collagen in the stroma which is believed to underlie corneal transparency, is dictated by the anterior epithelium over a

relatively brief time interval during embryonic development. It also opens up the possibility that the three dimensional structure of many mesodermal derivatives may be determined early in development by epithelial sheets. Another project established that even when the corneal epithelium is performing this specialized function it can be reprogrammed to differentiate in totally different ways. The anterior epithelium has been induced to form feathers or scales when it is confronted by competent mesenchyme of suitable origin.

The nucleic acid and protein chemistry of the embryonic lens are being studied by techniques which have been adapted to the minute amounts of tissue available at early embryonic stages. It has been found that the in vitro differentiation of lens epithelial cells into lens fibers which is produced by the addition of protein to the culture medium is accompanied by a conversion of 45s to 28s and 18s RNA. This finding helps to explain at the molecular level what happens when these cells differentiate into fibers. Studies are also continuing in vivo on the reconstruction of lenses from lens epithelium. One project seeks to determine whether primitive, uninduced ectoderm can form a lens when it replaces the lens of a well developed eye. Another project has confirmed that the mechanisms which control the orientation of the lens during its development can operate across taxonomic lines.

An analysis of the ways in which the eye establishes orderly central connections has gone forward in two ways. It has been demonstrated that the developing neural retina of the chick embryo is not specified as to its laterality before the third day of incubation. In a separate study a technique is being developed to ablate the isthom-optic nucleus during early development, thus preventing development of afferents to the neural retina. This approach may tell us a good deal about the function of retinal afferents.

Physiologic and pharmacologic studies on aqueous humor dynamics were continued in the Section on Pharmacology. The cat served again as the experimental animal. The rate of decay of I^{125} tagged albumin, which had been injected into the anterior chamber, was determined by the use of a gamma probe placed in front of the cornea of the anesthetized animal. The Kout could be recorded by continuous monitoring. The permeability of the blood-aqueous barrier tested by means of systemically administered inulin-C^{14} was not altered for about 2 hours after

puncturing the cornea with needles. Later the barrier allowed entrance of the labeled inulin and then, the linear relation between the intraocular pressure and the turnover rate of labeled albumin was disturbed. Aqueous humor formation was variably affected by low doses of the metabolic inhibitors, acetazolamide and oubain. At higher doses both drugs decreased the turnover rate. The injection of l-noradrenalin into the anterior chamber lowered the turnover rate in only half of the experiments.

Investigations on the role of the vasculature of the eye in controlling the intraocular pressure made use of the arterially perfused iris preparation as an experimental model. The beta-adrenergic blocking agents, pronethalol and propranalol, exerted an inhibitory effect on vasoactivity. The mechanisms are not clear. The previously described constriction of the iris artery of the cat by antiglaucomatous drugs was impeded by the use of beta-adrenergic blockers. These observations may be helpful in the analysis of the mode of action of drugs through effects on vascular responses or on other mechanisms.

In the general area of histopathology several studies relating to clinical material were carried out. Among these is included the first description of the ocular pathology in a well-documented case of a-beta-lipoproteinemia (Bassen-Kornzweig syndrome).

Previous studies of retinal and uveal inflammation produced in monkeys by sensitization to retinal antigen led to an attempt to produce a similar allergic response in rats. This more convenient laboratory animal, however, failed to develop either clinical or histological signs of ocular inflammation following inoculation of homologous retinal tissue.

Cellular proliferation and differentiation in the lens epithelium is under examination from several different aspects. Efforts to establish conditions for culture of the rat lens have been successful to the extent that all explanted lenses survive and demonstrate relatively active metabolism during culture periods of up to seven days. However, even in the unsupplemented synthetic medium there is a stimulation of cell-division in the normally non-proliferating central region after 48 to 72 hours of culture. Addition of insulin to the medium accelerates and increases the burst of mitotic activity. Hydro-cortisone has no effect on this system.

Cell cultures of trout lens epithelium were established and produced an extra-cellular, PAS-positive, fibrous substance assumed to be lens capsule material. This is the first observation of formation of capsule substance by isolated lens epithelial cells. A previous study of the transformation of ocular tissues by oncogenic viruses was extended to include the lens epithelium. Hamster lens epithelial cells infected with simian virus 40 underwent transformation in vitro and produced tumors when injected into homologous hosts. The cytological characteristics of the tumor derived from this pure cell type suggested that the SV40 genome is important in determining morphology. The study also demonstrated that the lens epithelium, a cell population with no apparent malignant potential under natural circumstances can undergo neoplastic transformation after oncogenic virus infection.

Dibromomannitol (DBM), a new agent for the treatment of chronic myelogenous leukemia, has been shown to profoundly affect the proliferative capacity of the lens epithelium. This drug is structurally related to myleran which is also used in the treatment of chronic myelogenous leukemia and is cataractogenic in rats. When DBM is fed to rats continuously the lens epithelium shows progressive injury with eventual depletion of the population and total loss of the germinative zone and differentiating cells. This drastic loss of cells occurs in the presence of a maintained increase in the number of mitoses. Anterior cortical opacities have been observed in these animals. DBM appears to resemble myleran in its ability to damage the lens epithelium and to induce cataract under experimental conditions, but differs from myleran in the manner by which it interferes with the cell cycle.

Induction of cataracts by streptozotocin, a new diabetogenic agent, has been studied and compared with experimental cataracts developing in alloxan diabetes. Streptozotocin was effective in producing sustained elevations of blood sugar levels without loss of animals due to toxicity. Lens opacities developed rapidly and resembled alloxan induced cataracts in clinical appearance.

Clinical Investigations

An analysis of the electroretinograms (ERG) of patients with large chorioretinal scars and those with certain hereditary retinal diseases has shown that the amplitude and implicit time of the ERG responses behave independently in these conditions.

In circumscribed chorioretinal destruction response amplitude is reduced but there is no change in implicit time. However, the reduction of response amplitude in hereditary disease can be accompanied by changes in implicit time, and in some degenerations the changes in implicit time appear before the decrease in amplitude. It is suggested that the slowed response reflects an abnormality of the receptor systems involving the entire retina.

Two types of hereditary retinitis pigmentosa have been extensively studied. Marked changes in the ERG were found to precede any ophthalmoscopically visible abnormalities in a family with the sex-linked form of the disease. Support for the Lyon hypothesis is provided by the finding that carrier females show loss of function only in limited areas of the retina while the males are extensively affected. A family with autosomal dominant retinitis pigmentosa showing reduced penetrance has also been investigated. The ERG's of affected members in an early stage of the disease demonstrated delayed and reduced rod responses similar to those seen in dominant retinitis pigmentosa with complete penetrance. However, the cone responses were delayed even though normal in amplitude. This cone abnormality has not been found in dominant retinitis pigmentosa with complete penetrance.

The elaborate techniques available in the Branch for studying degenerative disorders of the retina explain the numerous referrals of patients for examination and evaluation.

Admissions of patients with various forms of uveitis still lead the census in the nursing unit. The extent of laboratory studies carried out on such patients has no parallel in other eye departments but the yield of information on possible causes of the disease remains meagre.

The chemotherapeutic regimen for infections presumably due to Toxoplasma gondii often gave gratifying results. In this report period further attempts were made to treat inflammation of the uvea with immunosuppressive or antitumor agents in addition to the routine steroid therapy. Methotrexate has been tested again in some patients, but cyclophosphamide and azothioprine have been used more frequently. There was no clear cut evidence for superior effectiveness of the new compounds on the course of the disease in the majority of cases with severe forms of uveitis of the recurrent type, and prolonged observation periods are required before the usefulness of the drugs can be judged.

Several immunological studies were and are being carried out to gain some insight into the mechanism of drug action and to strenghten the rationale for the preferential administration of one or other immunosuppressive agents. Quantitation of the immunoglobulins in the serum, tests to define the primary response to different antigens, and examination to determine the immunosuppressive action on antibody concentration, were carried out prior to and after drug administration. The delayed hypersensitivity reaction was studied with several methods and examination of the anti-inflammatory response at the cellular level after the systemic use of antimetabolites was continued by means of the skin window technic described last year.

It was intended to counteract signs of the rejection phenomenon by immunosuppressive drugs in patients with homologous corneal transplants referred to the Branch for this purpose. Hyperemia in the anterior segment was decreased, sometimes dramatically, but other effects were questionable. In view of the advanced stage of the rejection syndrome in the few patients studied, no conclusion can be drawn at present on the benefits of such treatment. In all the studies with antitumor or immunosuppressive drugs a strict dose schedule was employed to avoid systemic side effects of the new therapeutic agents.

A somewhat questionable side effect was observed clinically in a study of patients treated for myelogenous leukemia. Myleran and the new compound dibromomannitol, administered for many years and so prolonging the patient's life, appeared to produce circumscribed opacities near the posterior pole of the lens, but it could not be excluded that the disease itself was the cause of the lenticular change. However, experiments on rats have shown in the past the cataractogenic properties of myleran and DBM.

The measurement of the episcleral venous pressure with a new technique described last year and its relation to the pathologic elevation of intraocular pressure was extended to systematic examination of patients with glaucoma but no new cases with idiopathic increase of the episcleral venous pressure and glaucoma were discovered. No relation between the two pressures could be discovered when various drugs were instilled in suitable patients. Measuring the pressure in the episcleral arteries with the new methods was not considered preferable to the older technique of dynamometry.

Careful titration of the response of the intraocular pressure by the concentration and timing of instillation of antiglaucomatous medication was successful in most instances in providing control of the IOP within the safety zone without necessitating surgical intervention.

The description of eye manifestations of a rare systemic disease in children, the Wiskott-Aldrich syndrome, emphasized the frequent occurrence of severe blepharoconjunctivitis sometimes associated with molluscum contagiosum and local or systemic herpes simplex infection. Ophthalmologists should be aware that such ocular changes might point to the immunological mechanism of a severe systemic disease.

The study of cystinosis, an inherited disorder of amino acid metabolism, was significantly expanded with the aim of distinguishing between the benign and nephropathic forms of cystinosis. Biochemical examinations revealed that the plasma concentration of the amino acid was lower in cystinosis patients than in normal controls. On the other hand the free cystine in leukocytes and fibroblasts was about 100 times higher in the nephropathic patients and about 50 times higher in patients with the benign type of cystinosis than in controls. The intracellular cystine cannot sustain normal metabolism of these cells when they are cultured in cystine-free medium. In another part of the study intracellular crystals were identified electronmicroscopically in biopsy material from patients with either type of the disease. Fibroblasts and histiocytes contained polymorphous crystalline structures. It is assumed that the crystals first aggregate in organelles which resemble lysosomes. The deposits are similar in both the benign and the nephropathic forms of the condition. It is possible that the inherited disease stems from a defect of cystine metabolism in these organelles.

The research activity of the branch is reflected by the publication or acceptance of approximately 36 manuscripts during the past year. Thirty-three projects are reported of which twelve are new. Ten of the studies have been completed or terminated.

Serial No. NDS(I)-56 O/OPS 300(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Design and Construction of Ophthalmic Instruments; Research in Psychophysical Methods of Evaluating Vision.

Previous Serial Number: SAME

Principal Investigator: Ralph D. Gunkel, O.D.

Other Investigators: Peter Gouras, M.D.
Richard Brubaker, M.D.

Cooperating Units: None

Man Years

Total:	0.6
Professional:	0.6
Other:	0.0

Project Description:

Objectives: Efforts are made to secure maximum effectiveness of instruments, techniques and ideas from whatever source for use in clinical and experimental work relating to ophthalmology. Modifications of conventional instruments and design and construction of new ones is often warranted.

Methods Employed: Frequent additions and modifications are needed in the apparatus used in psychophysical and electrophysiological studies of the eye. Clinical and Research Associates often require new or different instruments in their study projects, and occasional repairs must be made due to use or misuse of even the conventional instruments.

Major Findings: An oil-driven device with attachment to the skull was designed and constructed for advancing microelectrodes into the cerebral cortex of the monkey for the work of Dr. Gouras. Other electrodes and electrode holders of different form were made for the work of Dr. Finkelstein and Mary Hoff.

The animal board and accompanying microscope mount in Dr. Gouras's apparatus were redesigned to provide greater maneuverability.

Several types of optical apertures, grids, and light patterns were designed for projection into the monkey's eye for Dr. Gouras's cortical experiments.

Some improvements have been made in the model eye, including the use of a soft plastic for the vitreous body, which permits the demonstration of "scleral depression" in ophthalmoscopy. Some models, both whole and sectional, have been made for realistic demonstration of several pathologies. These have been used primarily in the teaching project of Mr. Bartner and Dr. Paton in the Wilmer Clinic at Johns Hopkins University.

Infra-red light converters were fitted to stereo microscopes to facilitate dissection and manipulation of continuously dark-adapted retinas in Dr. Finkelstein's work with single rods and cones. Some assistance was also provided toward his attainment of a vibration-free operating stage and symmetically-drawn micro-electrodes.

Construction was begun on a major modification for the ganzfeld stimulator for clinical electroretinography. This device would provide for quick changes of both color and brightness from outside the electronic shield. This information would be automatically code-imprinted on each ERG record, saving time here and also in subsequent identification of records. The double saving in time during the test implies a consequent improvement in quality, since the more quickly certain types of patient-related data can be obtained, the more reliable and artifact-free it becomes.

A special variable background light for electroretinography was constructed in the ganzfeld for determining the rod saturation level in cone-free patients.

The Polaroid camera adapter was redesigned to include a shutter as well as the enlarging lens. In addition to taking large retinal photographs, with a simple change in the lens position the camera back can be used on the Zeiss slit lamp and on the Zeiss operating microscope.

Testing of color vision, dark adaptation, and retinal thresholds was continued, data being obtained on about 280 patients during the year.

Several ideas for instruments have been explored with Dr. Brubaker. One of the most promising appears to be a design for an instrument for measuring flare, rather than estimating it on the 1+ to 4+ scale. This device would be attached to and used in conjunction with the Goldmann slit lamp.

Significance to Bio-medical Research and the Program of the Institute: Three papers published in the Archives of Ophthalmology with Dr. Eliot Berson as chief author and one delivered at a meeting dealt extensively with data obtained in the dark adaptation and retinal threshold tests.

Some of the widely acclaimed research efforts from the Branch has resulted from the electro-physiological studies of Dr. Peter Gouras, using optical and other devices designed or modified for his specific purposes.

Frequent contributions in the form of apparatus, repairs and advice have contributed substantially to the efficacy and continuity of both the clinical and laboratory program.

Proposed Course of Project: It is proposed that this project be continued in its present flexible form.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 0/OPS 1012(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electrophysiologic and Psychophysical Studies of Retinal Degenerations

Previous Serial Number: SAME

Principal Investigators: Peter Gouras, M.D.
Daniel Finkelstein, M.D.
Richard Einaugler, M.D.

Other Investigators: Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years

Total:	1.8
Professional:	1.3
Other:	0.5

Project Description:

Objectives: To study retinal degenerations in man with electrophysiological and psychophysical methods.

Methods Employed: Dark adaptation and color vision testing, retinal profiles, perimetry, electroretinography and electrooculography are used to study patients and their families. In addition, retinal degenerations have been studied with fluorescein fundus photography.

Patient material: The subjects are either outpatients or are admitted to the in-service unit; they are referred from other institutes of NIH and from private ophthalmologists. Normal subjects were obtained from the Normal Control Program of NIH.

Major Findings: Changes in the electroretinogram (ERG) have been studied in patients with large chorioretinal scars and certain hereditary retinal diseases. Amplitude and implicit time of the ERG responses are shown to behave independently of one another in these conditions. Chorioretinal destruction produces a reduction in amplitude but no change in implicit time. Hereditary disease leads to reduction in amplitude which can be accompanied by changes in

implicit time. Some degenerations produce changes in implicit time before changes in amplitude. The delays in implicit time can be best explained by an abnormality of the rod and/or cone receptor system involving the entire retina. Alterations of implicit time due to the intensity and area of light stimulation and the state of retinal adaptation are considered.

Psychophysical and electrophysiological testing have been used to examine a family with sex-linked retinitis pigmentosa. Marked changes in the electroretinogram (ERG) precede any abnormalities visible with the ophthalmoscope. Both cone and rod responses are reduced in amplitude and delayed in implicit time. Cone responses are detectable after rod responses have disappeared. When rod function can be measured, scotopic luminosity curves and ERG responses to scotopically balanced lights indicate a normal rod action spectrum. The disease in males is widespread, whereas carrier females show loss of retinal function only in limited areas. Comparison of findings in carrier females with those in affected males provides support for the Lyon hypothesis.

A family with autosomal dominant retinitis pigmentosa showing reduced penetrance has been described. Reduced penetrance is established in patients who transmitted the gene defect to their offspring but are themselves clinically normal and beyond the age of risk for the disease. The electroretinograms (ERG) demonstrate rod responses reduced in amplitude and delayed in implicit time at an early stage similar to those seen in dominant retinitis pigmentosa with complete penetrance. Cone responses are also delayed in implicit time even when response amplitudes are normal. This cone ERG abnormality has not been found in dominant retinitis pigmentosa with complete penetrance.

Significance to Bio-medical Research and the Program of the Institute: The underlying defects in retinal degenerations will be better understood through study of early or atypical cases and through genetic classification of various types. The finding of latency as well as amplitude changes in rod and cone components offers a new parameter in the consideration of these defects. Clarification of the primary defects in the rod and cone receptors is essential for defining the mechanisms of these degenerations.

Proposed Course of Project: Patients with various retinal degenerations will continue to be examined using the more sensitive electroretinography apparatus with special attention to early diagnosis in family studies and to atypical cases. Follow-up examinations will be done to gain information on the course of these degenerations.

Honors and Awards: None

Publications:

Berson, E., Gouras, P., and Hoff, M.: Temporal aspects of the electroretinogram. Arch. Ophthal. 81: 207-214, 1969.

Berson, E.L., Gouras, P., Gunkel, R.D. and Myrianthopoulos, N.C.: Rod and cone responses in sex-linked retinitis pigmentosa (II). Arch. Ophthal. 81: 215-225, 1969.

Berson, E.L., Gouras, P., Gunkel, R.D. and Myrianthopoulos, N.C.: Dominant retinitis pigmentosa with reduced penetrance (III). Arch. Ophthal. 81: 226-234, 1969.

Serial No. NDS(I)-63 O/OPS 1016(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electrophysiological Studies of Mammalian Retina

Previous Serial Number: SAME

Principal Investigators: Peter Gouras, M.D.
Daniel Finkelstein, M.D.
Mary Hoff, A.B.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.6
Other:	0.3

Project Description:

Objectives: To determine the cells responsible for generating the electrical activity of the retina and to understand how visual information is coded.

Methods Employed: Dr. Gouras has been introducing fine glass micropipette electrodes into the retina and brain of anesthetized monkeys in order to study the responses and receptive field organization of single neural elements. Small spots of light obtained from a double beam system can be focused and observed on the retinal surface by means of a modified fundus camera. The energy, wavelength, area, timing and retinal position of both beams can be independently controlled. Electrodes have also been introduced into the optic tract in order to stimulate retinal ganglion cells antidromically.

Miss Hoff and Dr. Gouras have been employing a perfusion system for maintaining the isolated cat eye alive while electroretinograms and optic nerve responses of the retina are recorded.

Dr. Finkelstein employs an infra-red optical system in order to observe single vertebrate photoreceptors in the dark and impale them with fine microelectrodes.

Major Findings: Dr. Gouras's previous work on the monkey has shown that each of the three cone mechanisms of primate vision, known by psycophysical methods and by microspectrophotometry, can also be identified in single retinal ganglion cells. Depending upon their receptive field organization, there now appears to be at least 14 varieties of retinal ganglion cells in the monkey, which can be subdivided into 7 on-center and 7 off-center groups. In each group there are phasically and tonically responding cells. Phasic ones are more common away from the fovea and receive excitatory and inhibitory signals from both red and green sensitive cones as well as from rods; signals from blue sensitive cones have not been detected in these cells. Tonic cells are more common near the fovea. They receive signals from only one cone mechanism in the center of their receptive field and antagonistic signals from another cone mechanism in the periphery of their receptive field. Therefore these cells show obvious opponent color responses. Tonic cells conduct impulses more slowly than phasic cells and therefore must be smaller cells. The foveal concentration of the single cone mechanism in the center of their receptive field and their small size suggest that tonic cells may belong to the midget cell system first described anatomically by Polyak.

Miss Hoff and Dr. Gouras have succeeded in recording normal ERG and optic nerve responses and some S-potentials in the isolated perfused cat eye, maintained alive for more than 10 hours often its removal from the animal. Such an eye shows normal dark-adaptation after very strong bleaching light indicating that visual pigment regeneration also takes place.

Dr. Finkelstein has succeeded in introducing fine electrodes into single vertebrate photoreceptors isolated from the remainder of the retina. So far the membrane potentials of these cells are positive internally and have not shown responses to light.

Significance to Bio-medical Research and the Program of the Institute: Such studies of retinal function at the cellular level should prove valuable for understanding vision and the pathophysiology of retinal disease.

Proposed Course of Project: To continue these projects along the lines already started.

Honors and Awards: None

Publications:

Gouras, P.: Identification of cone mechanisms in monkey ganglion cells. J. Physiol. 199: 533-547, 1968.

Gouras, P.: Vision. In Eggenberger, D. I. (Ed.): McGraw-Hill Yearbook, Science and Technology. New York, McGraw-Hill, 1969, pp. 344-346.

Serial No. NDS(I)-68 O/CB 1528(c)
1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Origin of Electrical Responses in Photoreceptor Cells

Previous Serial Number: SAME

Principal Investigators: Arnaldo Lasansky, M.D.
M.G.F. Fuortes, M.D.

Other Investigators: None

Cooperating Units: Laboratory of Neurophysiology, NINDS

Man Years

Total:	1.0
Professional:	0.6
Other:	0.4

Project Description:

Objectives: To investigate the location of the excitable membrane and the nature of the transducing mechanisms in visual cells.

Methods Employed: Electron microscopy. Electrophysiological recording combined with techniques to identify the position of microelectrodes.

Major Findings: The work has been completed by performing resistance measurements in leech visual cells. These measurements indicate that the channels connecting the vacuole to the outside fluid represent effective pathways for the receptor current, since their resistance--in spite of the junctional structures bridging their lumen--is only about three fold higher than it would be expected if they were filled by Ringer solution. It was also determined that following illumination the resistance measured from either the vacuole (8×10^6 ohms in the dark) or the cytoplasm (20×10^6 ohms in the dark) to the outside, drops to a value of 2×10^6 ohms.

Significance to Bio-medical Research and the Program of the Institute: These results provide a better understanding of the processes mediating visual excitation.

Proposed Course of Project: Project completed.

Honors and Awards: None

Publications:

Lasansky, A. and Fuortes, M.G.F.: The site of origin of electrical responses in visual cells of the leech, Hirudo medicinalis. J. Cell Biol. (In press).

Fuortes, M.G.F. and Lasansky, A.: Receptor potentials. In Reichardt, W. (Ed.): Proceedings of the Conference on "Processing of Visual Information". Varena, Italy, 1968, Academic Press (In press).

Serial No. NDS(I)-69 O/CB 1659(c)
1. Ophthalmology Branch
2. Section on Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Synaptic Contacts of Vertebrate Visual Cells

Previous Serial Number: NONE

Principal Investigator: Arnaldo Lasansky, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total	1.2
Professional:	0.6
Other	0.6

Project Description:

Objectives: 1) To investigate the fine structure of the synaptic contacts of visual cells; 2) To identify the cells that give origin to the post-synaptic processes.

Methods Employed: Electron microscopy associated, when necessary, with silver impregnations (Golgi method) and tracer experiments with electron-opaque markers.

Major Findings: There is already in the literature a considerable amount of information on the structural aspects of the invaginated or ribbon synapses of the visual cells. The initial aim of the present work was to extend those observations to the turtle retina, since this retina appeared to be a suitable material for microelectrode work, due to the relatively large size of its neurons. It soon became apparent, however, that additional junctional areas at the basal surface of cone pedicles exhibit structural features similar to those found at septate junctions in invertebrate epithelia. These basal junctions have received less attention in the past than the ribbon synapses; their resemblance to septate junctions may be of significance, since the latter are thought to represent areas of electrical coupling between cells.

Significance to Bio-medical Research and the Program of the Institute: It is hoped that the structural data will help to identify the mechanisms of

synaptic transmission in the retina. In addition, a better knowledge of the retinal networks may give the means to understand how visual information is processed in the retina.

Proposed Course of Project: 1) The permeability of the intercellular gap at the basal junctions will be explored by means of electron-opaque tracers. The structure of synapses known to be chemical or electrical will be investigated for purposes of comparison with the basal junctions. 2) The identity of the cells establishing basal or recessed junctions with the visual cells will be determined by examining Golgi preparations at the electron microscope level.

Honors and Awards: None

Publications:

Lasansky, A.: Basal junctions at synaptic endings of turtle visual cells. J. Cell Biol. 40: 577-581, 1969.

Serial No. NDS(I)-63 O/OCH 1017(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Physical Chemistry of Corneal Collagen

Previous Serial Number: SAME

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To determine the physical and chemical parameters related to the molecular structure of corneal collagen with the view of examining them for significance in the morphology of the cornea.

Methods Employed: Collagen has been extracted from bovine corneas and has been purified by conventional means. Ultracentrifugal analysis has been used to attempt to obtain the distribution of the molecular weights of collagen polymers.

Major Findings: No significant new information has been obtained in this research. The work which has been done on the development of the analysis of multi-component systems has been applied to the analysis of lens proteins as these represent a simpler system better suited for this stage of the development of the methods of analysis.

Significance to Bio-medical Research and the Program of the Institute: This project represents a continued study of the relationship of the molecular structure of certain proteins of ophthalmic origin to the structure and function of the tissues of their origin. It provides a base for comparative studies of different collagens in the eye, and for studies of the role of collagen in corneal development and in corneal pathologies.

Proposed course of Project: Continued studies on molecular weight distribution on both native and denatured collagen are planned, since it would appear that this information might be of significance in understanding the process of aggregation of collagen monomers into polymers which ultimately form the collagen fibril. While the development of methods of analysis of systems of this type has been slower than was anticipated, the present techniques appear to be suitable for the analysis of the simpler system obtained with denaturation of the collagen polymers into a limited number of subunits. It is possible that such an analysis will be of use in approaching the more difficult problem of analysing the native polymers.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-65 O/OCH 1210(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Chemistry of Rhodopsin

Previous Serial Number: SAME

Principal Investigator: Marc S. Lewis, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description:

Objectives: To study the structural and functional aspects of the bovine rhodopsin molecule.

Methods Employed: Rhodopsin was extracted from outer segments of dark-adapted bovine retinas with 1% Emulphogene BC-720, a non-ionic detergent (alkoxy-polyethylene-ethanol) and was purified by chromatography on ECTEOLA-cellulose and calcium phosphate columns. The results of spectrophotometry, polyacrylamide gel electrophoresis, and analytical ultracentrifugation were used as criteria of purity. Succinyl rhodopsin was prepared by adding succinic anhydride to a solution of rhodopsin in detergent with the pH maintained constant, and then removing the detergent by dialysis. Delipidated rhodopsin was prepared by treatment with a chloroform-methanol mixture. Molecular weight studies were performed on a variety of rhodopsin samples by means of the high-speed equilibrium technique in the ultracentrifuge.

Major Findings: Rhodopsin in detergent solution was found to exist as a rhodopsin-detergent complex. Equations were derived to describe this interaction in terms of assumed molecular weight and calculated partial specific volume for the rhodopsin and a determined partial specific volume for the detergent. By doing similar experiments using D_2O instead of water for all of the solutions, it was possible to calculate the molecular weight of the complex and also the molecular weight of the rhodopsin in the complex. Under these

conditions, rhodopsin was found to have a molecular weight of 35,000. It was also found that bleaching had no apparent effect on the molecular weight of the rhodopsin-detergent complex if it was in a 1% detergent solution, but that a significant effect was observed in 0.1% detergent. Studies on succinyl rhodopsin showed that it was a dimer with a molecular weight of 71,000 in the absence of detergent. Delipidated rhodopsin was initially studied using 2-chloroethanol as a solvent, but it was found that the HCl released by the degradation of the 2-chloroethanol hydrolysed the rhodopsin. It was found possible to avoid this by the addition of 10% pyridine as a buffer. The delipidated rhodopsin was studied in this mixture and also in a similar mixture of 2-bromoethanol and pyridine. Because of the markedly different densities of these two solvents, it was possible to obtain good values for both the molecular weight and the partial specific volume of the delipidated rhodopsin in these solvents. The molecular weight was found to be 34,000, which is in very good agreement with the value for rhodopsin in a complex with detergent. The partial specific volume was found to be 0.733 as compared to 0.737 which was calculated from the amino acid composition.

Significance to Bio-medical Research and the Program of the Institute: These studies represent the first comprehensive study of the molecular weight of this very important lipoprotein that does not involve assumptions which limit the validity of the results. The results obtained here infer that there is a hydrophobic region on the surface of this molecule which is responsible for the dimer formation of succinyl rhodopsin, and which is also probably of significance in the incorporation of the rhodopsin in the rod outer segment membrane. The changes in the molecular weight of the rhodopsin-detergent complex in solutions of low detergent concentration implies that this might be a useful approach to studying the configurational changes accompanying bleaching.

Proposed Course of Project: Further studies of various rhodopsin preparations in various solvent systems with particular emphasis on means of studying the configurational changes accompanying bleaching.

Honors and Awards: None

Publications:

Shichi, H., Lewis, M.S., Irreverre, F., and Stone, A.L.: Biochemistry of visual pigments. I. Purification and properties of bovine rhodopsin. J. Biol. Chem. 244: 529-536, 1969.

Serial No. NDS(I)-65 O/OCH 1217(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Physical Biochemistry of Model Gel Systems

Previous Serial Number: SAME

Principal Investigators: Marc S. Lewis, Ph.D.
Ralph J. Helmsen, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: To study the physical and chemical parameters of model systems which are pertinent for transparency and opacity of gel systems. In particular, the objectives of this study are to isolate and characterize the water-soluble proteins of lenses of embryonic and newly hatched chicks, to determine the quantity of each component, and to attempt to relate these parameters to the events of embryonic development.

Methods Employed: Lenses have been obtained from newly hatched chicks and from chick embryos at various stages of development. The water-soluble proteins were extracted and subjected to a variety of fractionating and analytical techniques, primarily involving precipitation at various pH values, Lowry and spectrophotometric protein determinations, gel electrophoresis, and analytical ultracentrifugation.

Major Findings: While classical precipitation techniques are satisfactory for the isolation of water-soluble lens proteins, they do not permit adaquate determination of the relative quantities of the various components. Step-wise precipitation obtained by lowering the pH in small increments does not give suitable resolution of the components. Gel electrophoresis was found to be useful for identification and for determining the number of components in a

particular fraction, but did not permit the desired degree of quantitation of relative amounts of the individual proteins. The most promising results to date have been obtained by ultracentrifugal analysis. A new system for the analysis of multi-component solutions has been devised and is being evaluated. This system involves the resolution of the sums of different exponential concentration distributions obtained at different rotor speeds. While this appears to improve the precision obtained as compared to such a system at a sin rotor speed, it has also greatly increased the complexity of the analysis. The apparent values of the molecular weight of the two lightest proteins from water extracts of the lenses of newly hatched chicks are 113,000 and 270,000, assuming a partial specific volume of 0.72. Each of these values appears to be approximately double the usual value of the molecular weight of beta-crystallin (50,000) and of delta-crystallin (150,000), suggesting the possibilities that dimers are being observed here, or that the lower molecular weights might be artifacts of preparation. Other independent evidence will be required to evaluate either of these possibilities.

Significance to Bio-medical Research and the Program of the Institute: These studies are of significance in relating the time of appearance, the relative quantities, and possible changes in the properties of the water-soluble lens proteins with the development of the embryonic lens. In addition, the analytical techniques developed for this work would appear to be of significant value for other studies involving multi-component systems.

Proposed Course of Project: The ultracentrifugal analyses of the lens protein solutions will be continued and will include the analysis of lenses from all stages of embryonic development where it is possible to obtain samples In addition, preparative density gradient ultracentrifugation will be tried as a means of effecting separation and quantitation of the various proteins. Immunological techniques and comparison of the physical and chemical properties of these proteins and of lens proteins prepared by classical methods will be used for purposes of identification.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 O/OCH 1433(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Chemistry of the Vitreous Body

Previous Serial Number: SAME

Principal Investigator: Ralph J. Helmsen, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.7
Other:	0.0

Project Description:

Objectives: To determine physical and chemical parameters related to the molecular structure of vitreous macromolecules with the view of examining these for significance in the morphology of the vitreous body.

Methods Employed: Two major lines of investigation have been undertaken: isolation of distinct proteins from the vitreous of cattle and immunological examination of these proteins to determine the sites of their origin. Ammonium sulfate precipitation and column chromatography were used to fractionate and isolate the bovine vitreous proteins. Analytical ultracentrifugation, intrinsic viscosity, optical rotatory dispersion, amino acid analysis and carbohydrate analysis were used for characterization. Immunological comparison between the vitreous proteins and similar proteins derived from other tissues were effected through immunodiffusion and precipitation techniques as well as by immuno-electrophoresis. The latter procedures will also be employed, in part, to distinguish the isolated protein from paraproteins which may occur in the vitreous of animals suffering from certain eye abnormalities.

Major Findings: Soluble proteins and glycoproteins of the vitreous were separated from contaminating mucopolysaccharides on DEAE-Sephadex after preliminary dialysis against neutral 0.005 M phosphate buffer. The major ultraviolet absorbing peak obtained from chromatography was fractionated according to the procedure of Weimer and associates for serum orsomucoid. Material

isolated as a precipitate from the pH 3.7 filtrate was dialyzed against neutral 0.2 M phosphate and examined in the analytical ultracentrifuge. Preliminary analysis of the fraction by sedimentation equilibrium revealed a major component of approximately 55,000 (96%) and a minor component of approximately 295,000 (4%). Further purification of the lighter of the two components is presently being pursued by application of the technique of ultrafiltration through a membrane in the presence of a centrifugal field.

Significance to Bio-medical Research and the Program of the Institute: These projects represent continued study by this investigator into the interrelationship of the molecular structure of the macromolecular constituents of the vitreous body to the structure of this avascular connective tissue. It provides the base for determination of possible alterations in the tertiary structure of these macromolecules in various disease states affecting the vitreous.

Proposed Course of Project: In addition to further characterization of the two components already discussed under Major Findings, pertinent modifications will be made from time to time in the chromatographic procedures presently being employed to make them feasible for clinical application. New procedures will be sought to isolate additional protein macromolecules from bovine vitreous as well as the corresponding tissue from other animals. Each distinct constituent thus obtained will be identified, if possible, by the immunological techniques referred to in the Methods Section. The ultimate goal of this systematic attack on the structure of the individual proteins of the vitreous is to furnish needed information in order to construct a working model to explain the gel-liquid structure of this connective tissue. The studies conducted on chickens with photo-induced enlargement of the vitreous and buphthalmos will be resumed as soon as new animal facilities are available.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 O/OCH 1530(C)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Biological Oxidations in the Retinal Pigment Epithelium

Previous Serial Number: SAME

Principal Investigator: Hitoshi Shichi, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: The retinal pigment epithelium is of major significance in the metabolism of the retina and thus has a vital role in the visual process. Previous work has indicated that the pigment epithelium microsomes contain a haemoprotein component which apparently functions as the terminal oxidase for oxidation of the reduced pyridine nucleotides. The haemoprotein has been solubilized, pruified and shown to be spectrally identical with haemoprotein 559 previously characterized in heart microsomes, and with P420, a modified form of a haemoprotein (P450) which is believed to be involved in microsomal mixed function oxidase reactions. The haemoprotein (P420) shows a peculiar spectral property, i.e., pH-dependent hyperchromism at the α-band of the reduced form. The objective of this project is to investigate the structure of the chromophore of the haemoprotein in the hope of elucidating the mechanism of hyperchromism and, preferably, the chemical nature of P450. This is a part of the projects proposed in the last annual report, i.e., physico-chemical studies of the microsomal haemoprotein (P420).

Methods Employed: (1) The endoplasmic reticulum of the retinal pigment epithelium was collected as microsomal particles by differential centrifugation of the tissue homogenate in sucrose-phosphate buffer. A haemoprotein component in the microsomes was solublized with trypsin and purified by fractionation with ammonium sulfate by chromatography on Sephadex. (2) Haemochromes of polypeptides were prepared by mixing polypeptides with protohaem IX and reducing

the complexes formed with sodium hydrosulfite.

Major Findings: (1) Of poly-L-glutamic acid, poly-L-phenylalanine, poly-DL-tryptophan, poly-L-tyrosine, poly-L-lysine and poly-L-histidine, only the latter two compounds produced haemochromes which spectrally resembled P420. Polyhistidine haemochrome was stable at pH values above 6 but polylysine haemochrome was stable only above pH 11. The results of model experiments thus suggested histidine as a possible amino acid involved in haem binding. (2) The fact that succinylation of all amino groups (ninhydrin-positive groups) of P420 did not affect the hyperchromism of the haemoprotein while diazotization of histidine residues in P420 with diazonium tetrazole resulted in the gradual loss of the α-absorption band supports the possibility of histidine rather than lysine as the haem binding group. (3) The addition of poly-L-tyrosine to poly-L-histidine haemochrome resulted in a decrease in intensity of the α-band, the pH-dependent pattern of which resembled closely the comparable pattern demonstrated with P420. From the comparison of molar extinction coefficients of polyhistidine haemochrome at different pH and at different polytyrosine concentrations, it was evident that the real nature of hyperchromism was hypochromism at low pH caused by polytyrosine. (4) From these results, tyrosine as well as histidine residue was suggested as possibly involved in the hypochromic, rather than hyperchromic, phenomenon of P420.

Significance to Bio-medical Research and the Program of the Institute: The haemoprotein solubilized from pigment epithelium microsomes is spectrally identical with P420, a modified form of a cytochrome (P450) involved in microsomal mixed function oxidase reactions, but it appears to be present as a native protein in some cellular organelles, e.g., heart microsomes, neutrophilic granules of leucocyte and mitochondria of cancer cells. The possibility has previously been suggested that the haemoprotein may be involved in the microsomal electron transfer system of pigment epithelium responsible for melanin formation and in drug metabolism. In order to understand the mechanism of the biological oxidation reactions, it is important to clarify the chemical nature of the chromophore of the haemoprotein directly involved in these reactions. The structure of the haem binding site of the protein, as suggested by the present investigation, may provide a clue to characterize the chromophore structure of P450 and to synthesize chemically an artificial terminal oxidase for the drug metabolism which can be used under certain pathological conditions of pigment epithelium.

Proposed Course of Project: (1) Study the metabolism of tyrosine and other intermediates for melanin synthesis by the microsomes and the possible role of P450. (2) Definition of the individual enzymes and mechanism of reactions.

Honors and Awards: None

Publications:

Shichi, H.: Microsomal electron transfer system of bovine pigment epithelium. Exp. Eye. Res. 8: 60-68, 1969.

Serial No. NDS(I)-69 O/OCH 1660(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Biochemistry of Visual Pigments

Previous Serial Number: NONE

Principal Investigator: Hitoshi Shichi, Ph.D.

Other Investigator: Filadelfo Irreverre, Ph.D.

Cooperating Units: Laboratory of Biophysical Chemistry, NIAMD

Man Years

Total:	0.8
Professional:	0.8
Other:	0.0

Project Description:

Objectives: (I) To study the amino acid sequence near the carbohydrate-protein linkage in a visual pigment, rhodopsin. (II) To study functional aspects of rhodopsin in the outer segments of retina photoreceptors.

Methods Employed: (I) Rhodopsin was extracted from outer segments of dark adapted bovine retinas with 1% Emulphogene BC 720, a nonionic alkoxypoly-(ethyleneoxy)ethanol, and purified by chromatography on calcium phosphate and ECTEOLA-cellulose. The purified pigment was shown to be homogeneous by chromatography on ECTEOLA-cellulose, polyacrylamide gel electrophoresis and spectrophotometry. The purified pigment was delipidated with chloroform-methanol and was chemically cleaved by treatment with cyanogen bromide to prepare a mixture of peptides. The peptides were then subjected to chromatography and high-voltage electrophoresis (peptide mapping). A carbohydrate-peptide separated in this way, was hydrolyzed and the amino acid composition of the peptide was determined with an automatic amino acid analyzer. (II) Outer segments of bovine retina were sonically disrupted to prepare membrane particles. Circular dichromism and infrared spectral measurements were made on the particles.

Major Findings: (I) A. The chemical cleavage of delipidated rhodopsin with cyanogen bromide gave rise to at least 8 peptides. The rhodopsin molecule was previously shown to contain 8 moles of methionine per mole. Since cyanogen bromide specifically cleaves peptide bonds involving methionine residue, the

detection of 8 peptides confirms the previous result of amino acid analysis. B. The preliminary analysis shows that the carbohydrate peptide contains aspartic acid (1), threonine (1), serine (3), glutamic acid (2), glycine (5) and alanine (1). The N-Terminal amino acid of this peptide appears to be glycine. (II) The outer segment membrane of retina was disintegrated by soni cation into fine particles whose diameter range from 50 to 200 Å. Circular dichroism measurements showed that the particles, like purified rhodopsin, contained approximately 60% helical structure. The particles, however, did not undergo conformational change after irradiation, thus resembling the oute segment membrane. A single particle (d=50Å) was calculated to contain one rhodopsin molecule which has visible spectra identical to those of outer segment. Like outer segments, the particles possess a K^+ -Na^+ dependent ATPase activity.

Significance to Bio-medical Research and the Program of the Institute: Rhodopsin is a visual pigment which plays a crucial role in the eye for the initiation of visual excitation for black white perception. The trigger reaction for neural impulse is believed to be generated by the light-induced change in the pigment. Except for the photoisomerization of 11-cis retinal to all trans retinal, we know very little of the nature of the change which is supposed to take place in the protein moiety of the pigment. The current finding on the chemical structure of the carbohydrate binding site of the pigment is only a preliminary step to elucidating the complete structure of the pigment. Studies on the isolated pigment may have of limited physiologic significance. Therefore, attempts were made to prepare very small fragments of the outer segment membrane of retina. The finest particle of outer segment obtained by sonic disruption appears to contain only one rhodopsin molecule and still possess membrane properties. The chemical characterization of the particle should provide useful insight into the structure of the membrane of the discs in the outer segments where visual excitation is initiated.

Proposed Course of Project: (1) Elucidation of amino acid sequence around the carbohydrate-binding site of opsin. (2) Characterization of chemical nature of fine fragments of the outer segment membrane.

Honors and Awards: None

Publications:

Shichi, H., Lewis, M.S., Irreverre, F. and Stone, A.L.: Biochemistry of visual pigments. I. Purification and properties of bovine rhodopsin. J. Biol. Chem. 244: 529-536, 1969.

Serial No. NDS(I)-68 O/OM 1531(c)
1. Ophthalmology Branch
2. Section on Ophthalmic Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Synthesis of Sugar-Containing Polymers in Retina

Previous Serial Number: SAME

Principal Investigator: Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.4
Professional:	1.0
Other:	0.4

Project Description:

Objectives: To determine the kinetic properties and cofactor requirements of a mannose transfer enzyme system present in bovine retina which catalyzes the addition of mannose residues to endogenous pre-formed polypeptides. To determine the solubility characteristics of the products formed and to compare them with the products of the transfer of sialic acid by a similar system previously reported.

Methods Employed: Bovine retinas are homogenized and the mitochondrial-microsomal fraction is isolated by differential centrifugation. The transfer of sugars to this particulate fraction is measured as acid insoluble radioactivity after incubation with the radioactive sugar nucleotide. The products are identified as glycoproteins by their sensitivity to proteolytic enzyme digestion. Products are extracted from the particulate enzyme preparation with detergents and are partially purified by column chromatography on Sephadex.

Major Findings: 1) Mannose is transferred from GDP-Mannose to an endogenous acceptor with a Michaelis constant (Km) of 2μM for GDP-Mannose. 2) The transfer occurs over an extremely broad range of pH, 6.1 to 8.4, with a slight maximum at pH 7.8. The products across this range appear to be the same. 3) GTP stimulates the transfer by sparing GDP-Mannose from degradation. ATP and AMP, both common effectors of enzyme activity have no effect. 4) A chelating agent, EDTA, inhibits the enzyme, presumably by removing a divalent cation.

$MnCl_2$ restores the activity. $MgCl_2$ is only partly effective. 5) UDP-N-acetyl glucosamine also stimulates the transfer. The mechanism is not known. 6) The products of the transfer are firmly bound to the particulate enzyme preparatio but can be solubilized by detergents, in particular cetyltrimethylammonium bromide (CTAB). CTAB extracts passed over a column of Sephadex G-100 show a single radioactive peak which coincides with one of three radioactive peaks observed when a similar extract of an enzyme preparation labeled with radio-active sialic acid is passed over the same column. 7) Approximately half of the mannose-labeled product is digested by a proteolytic enzyme to fragments too small to be precipitated by acid and is therefore probably glycoprotein.

Significance to Bio-medical Research and the Program of the Institute: The enzyme systems necessary for the biosynthesis of glycoproteins are present in the retina. These systems have been demonstrated in homogenates and are known to be functioning in whole retinas. The glycoprotein products appear to be structural components of the cell membranes, in particular the outer segmen of the photoreceptors. Since these photoreceptors seem to be regenerated in a fairly short time (1-2 weeks in laboratory animals), defects in any of the enzymes involved in membrane synthesis might manifest themselves as progressiv degeneration of the rods. Detailed knowledge of these processes will permit testing in model systems.

Proposed Course of Project: To characterize further the products of the several transfer systems studied so far. To apply sophisticated cell fractio ation techniques in order to isolate the specific structures with which thes enzyme systems are associated. To study the incorporation of glucosamine into rhodopsin and the structural components of the photoreceptor cell. To study the control of these processes with emphasis on the effects of environmental conditions, especially light.

Honors and Awards: None

Publications:

O'Brien, P.J. and Muellenberg, C.G.: Properties of glycosyl transfer enzymes of bovine retina. Biochim. Biophs. Acta. 167: 268-273, (1968).

Serial No. NDS(I)-68 O/OM 1533(c)
1. Ophthalmology Branch
2. Section on Ophthalmic Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Protein Synthesis in the Retina

Previous Serial Number: SAME

Principal Investigators: Jacqueline J. Bungenberg de Jong, Ph.D.
Paul J. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.2
Professional:	1.0
Other:	0.2

Project Description:

Objectives: To study and identify the products of protein synthesis in the bovine retina, with emphasis on the proteins of the rod outer segments, in order to determine whether rhodopsin or its apoprotein can be synthesized by the retina when incubated as a tissue slice in vitro.

Methods Employed: Bovine retinas are isolated under dim red light in such a fashion that as little damage as possible is done to the tissues and that contamination with pigment epithelium is kept to a minimum.

The retinas are incubated in the dark under a continuous flow of O_2-CO_2 (95%-5%) in the presence of 0.5 µc ^{14}C-leucine per ml medium. The medium for these incubations consists of an isotonic Krebs-Ringer solution made 0.02M with respect to glucose to which are added 100 µg of casein hydrolysate per ml. Streptomycin and penicillin are present to prevent bacterial growth.

Incorporation of radioactive precursor amino acid into the protein of the whole retina and its subfractions is measured as acid precipitable, lipid solvent insoluble, radioactivity detected in a scintillation counter.

The subcellular fractions of the retina are isolated and purified by a combination of differential and density centrifugation.

In order to study whether rhodopsin has become labeled during incubation the following methods are employed:
1. The rod outer segments are treated with $NaBH_4$ in order to form a covalently bound N-retinyl opsin which has a specific absorption at 333 mμ. The radioactive rod outer segment proteins are solubilized in detergent solutions.
2. SDS (Sodium Dodecyl Sulfate) extracts of the proteins of rod outer segments and the other subcellular fractions of the retina are fractionated by column chromatography and polyacrylamide electrophoresis.

Major Findings: 1) Reduction of rhodopsin in the isolated outer segments with $NaBH_4$ is possible in the absence of detergents. The $\frac{A\ 280\ m\mu}{A\ 330\ m\mu}$ of such preparations in 1% SDS was between 5.3 and 7.4 in 5 different experiments and is comparable to that of preparations reduced by $NaBH_4$ in the presence of CTAB (Hexadecyltrimethylammonium Bromide). 2) The reduced rhodopsin in 1% SDS can be separated from other rod proteins by column chromatography on Sephadex-G-100. The $\frac{A\ 280\ m\mu}{A\ 330\ m\mu}$ of the rhodopsin peak decreases during two successive purifications to about 2. 3) The purification of rhodopsin can also be monitored using polyacrylamide electrophoresis. A system using 5% acrylamide in 0.1% SDS-tris buffer as support with 0.04 M tris-glycine buffer made to 0.1% with SDS as the developing buffer gives the best results so far. A solution of rhodopsin purified by column chromatography gives one major dark band and one minor light one on the gel after staining with Coomassie-Blue-Brilliant. 4) It has been found possible to reclaim rhodopsin from 1% SDS solution by Alcohol-Ether precipitation at 0°C. The resulting precipitate of denatured rhodopsin is soluble in 1% SDS without change in $\frac{A\ 280\ m\mu}{A\ 330\ m\mu}$ and its behavior on column chromatography and gel-electrophoresis is identical to that of SDS solutions of native rhodopsin. 5) When bovine retinas are incubated in vitro with ^{14}C-leucine, rhodopsin becomes labeled. About 25% of the total radioactivity of rod outer segment proteins is associated with rhodopsin as purified with column chromatography. The specific activity of the rhodopsin peak area of such a column, expressed as counts per minute in 1 ml column eluate/O.D. at 330 mμ increases from 88 to 300 between 1 and 2 hours.

Significance to Bio-medical Research and the Program of the Institute: The study of the synthesis of the apoprotein of rhodopsin may contribute to the knowledge and understanding of metabolic defects in rhodopsin biosynthesis and possibly explain some forms of functional impairment of the retina.

Proposed Course of Project: To study the synthesis of the proteins of rod outer segments of beef retina in vitro with emphasis on rhodopsin. To determine whether there is any effect on the rate of rhodopsin synthesis under dark adapted and/or bleached conditions or in the presence or absence of the pigment epithelium, whether this is present in suspension or interdigitated with the retina. To study the effect of the presence of vitamin A and its derivatives on the synthesis of rhodopsin.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-56 O/OPH 301(c)

1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Study on the Pharmacodynamics of Various Agents Affecting the Intraocular Pressure

Previous Serial Number: SAME

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.95
Professional:	0.45
Other:	0.5

Project Description:

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure with a view toward finding more effective compounds and to possibly further the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: All agents investigated were examined on the cat eye. At equilibrium, the rate of aqueous humor formation is equal to the rate of aqueous humor outflow (Kout). Kout was determined by measuring the rate of decay of iodine (I^{125}) tagged albumin placed in the anterior chamber using a gamma probe mechanically fixed immediately in front of the cornea. This technique allows for the continuous monitoring of Kout and for the rapid determinations of drug action upon it.

Major Findings: The use of the gamma probe to determine the rate of decay of intracamerally injected (I^{125}) albumin has been found to be an excellent method for the determination of aqueous humor turnover. Utilizing this technique together with the aqueous humor sampling method reported last year the following findings have been made:

1. Puncture of the cornea with needles for various experimental purposes has no detectable effect on the permeability of the blood-aqueous humor barrier for an initial period of 1½-2 hours. After this time the barrier becomes

permeable to inulin ^{14}C (MW 5200). The response is not due to duration of anesthesia. During the first period, a linear relationship is found between the IOP and turnover rate. This relationship is not seen at the later time interval.

2. The metabolic inhibitors acetazolamide and ouabain are being studied for their ability to lower the rate of aqueous humor formation. Our findings to date indicate that acetazolamide, in the clinically-used dosage range, can either decrease, increase or have no effect on the aqueous humor turnover ra in the cat. As the dosage level is increased, the incidence of eyes respondi by a decreased turnover rate is increased. Similar findings have been made by the use of ouabain.

L-norepinephrine, placed directly in the anterior chamber, also has unpredictable effects on the turnover rate, although in greater than 50% of the experiments the turnover rate is decreased.

Significance to Bio-medical Research and the Program of the Institute: The finding of a breakdown in the blood-aqueous humor barrier after 1½-2 hrs experimentation on the eye cautions on the acceptability of data obtained at later time intervals.

It has been accepted by many investigators that acetazolamide decreases the rate of aqueous humor formation by a dose-related inhibition of carbonic anhydrase in the ciliary body. In a similar way ouabain was believed to proportionally decrease inflow of aqueous humor by inhibiting the enzyme, Na-K ATPase. These two drugs, particularly at the lower dosage levels, are known to inhibit specific enzymes, yet the response on inflow is not necessarily one of inhibition but could be one of activation. This brings into sharper focus a previous report from this laboratory which indicates that a vasoconstrictive action of both these drugs could lower eye pressure by decreasing the eye bloo volume.

Proposed Course of Project: It is proposed to continue to measure the effect of various anti-glaucoma agents on aqueous humor inflow and to try to correlate these findings with changes of eye pressure. In addition it is planned to alter the arterial and venous pressures of the eye and to determine whether these changes have effects on the aqueous humor turnover rates.

Honors and Awards: None

Publications:

Macri, F.J.: Annual review of pharmacology of ophthalmic drugs. Arch. Ophthal. 80: 506-521, 1968.

Macri, F.J. and Brubaker, R.F.: Methodology of eye pressure measurement. Biorheology. (In press).

Serial No. NDS(I)-59 O/OPH 600(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Role of Vasculature in the Maintenance of Intraocular Pressure

Previous Serial Number: SAME

Principal Investigator: Frank J. Macri, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.05
Professional:	0.55
Other:	0.5

Project Description:

Objectives: To determine if and by what mechanisms the dynamics of the intraocular vasculature affect the eye pressure and to demonstrate the pharmacologic response of this vasculature to certain drugs.

Major Findings: We have reported earlier that some corticosteroids were capable of inhibiting, non-specifically, the vasoactivity of a number of agents on the isolated arterially perfused iris. We have found a similar type of action from the B-adrenergic blocking agents, pronethalol and propranolol. This inhibitory activity is probably not due to the B-blocking properties of these compounds since d-propranolol, a very weak B-blocker, shows no attenuation of activity on the isolated iris. A series of antiglaucoma agents which were known to constrict the iris artery (acetazolamide, ouabain, pilocarpine, epinephrine, norepinephrine) were all effectively inhibited from producing vasoconstriction by both of these B-adrenergic blocking agents.

Studies on the arterially-perfused posterior segments of cat eyes indicate that dexamethasone but not hydrocortisone or fluoramethalone produce vasoconstriction. From the data obtained, however, it could not be determined whether the vasoconstriction was on the arteriolar or venular branches.

Significance to Bio-medical Research and the Program of the Institute: The finding that B-adrenergic blocking agents will inhibit vascular responses on the iris artery by antiglaucoma agents is interesting. There has been considerable debate as to whether changes of the vasculature of the eye plays any significant role in lowering eye pressure in glaucoma. It may be that the B-adrenergic blocking agents may at last allow us to isolate, in vivo, that portion of drug activity which is due to vascular activity from those involvi enzymic and other mechanisms.

Proposed Course of Project: It is proposed to continue this project along the same lines as for the current year. The ability of B-adrenergic blocking agents to reverse the hypotensive action of glaucoma agents will be studied.

Honors and Awards: None

Publications:

Morgan, W.E. and Macri, F.J.: Vascular responses of the posterior segment of the cat eye. Arch. Ophthal. 79: 779-784, 1968.

Serial No. NDS(I)-67 O/OPH 1434(c)
1. Ophthalmology Branch
2. Section on Ophthalmology Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: A Study of the Buphthalmic Rabbit Eye.

Previous Serial Number: SAME

Principal Investigators: Frank J. Macri, Ph.D.
P.R.B. McMaster, M.D., LGAR, NIAID

Other Investigators: None

This project has been terminated.

Serial No. NDS(I)-69 O/EE 1661(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ocular Morphogenesis - Scleral Ossicles and Scleral Cartilage.

Previous Serial Number: NONE

Principal Investigator: A. J. Coulombre, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.8
Other:	0.2

Project Description:

Objectives: There are two general objectives: 1. To identify and characterize the mechanisms which control the size, shape and orientation of structures in the outer coat of the eye; and 2. to assess the morphogenetic role of collagen which is deposited early in the development of the eye.

Methods Employed: The methods include: microsurgery of chick embryos, mensuration (linear, planimetric, volumetric), chemical determinations (DNA, hydroxyproline, hexosamine) and electron microscopy.

Major Findings: The work on this project currently focuses on two undertakings:

A. Control of the growth and shape of the scleral cartilage of the chick embryo.

1. Surgical removal of the lens at five days of incubation prevents the accumulation of vitreous substance and leads to microphthalmia.
2. The scleral wall of such eyes is proportionately smaller in surface area than its normal counterpart, and slightly thicker. The mass of such sclerae increases subnormally.
3. A proceedure has been developed for cleanly removing the collagenous connective tissues which adhere to the cartilaginous sclera. This allows an accurate determination of the chemical constituents of the cartilaginous sclera. This, in turn, permits a more accurate replication of a previous experiment which showed an invariant relationship between DNA

and hydroxyproline in the developing eye walls of both normal and microphthalmic eyes.

B. Development of the overlap pattern among the scleral ossicles.

1. This expands and extends an earlier study which showed that in the chick embryo the scleral ossicles;

a. are foreshadowed by an equal number of conjunctival thickenings (papillae)

b. That the numbers of papillae or ossicles is usually fourteen, often fifteen and occasionally thirteen.

c. That removal of a papilla before a specific point in its development resulted in the failure of the corresponding ossicle to appear beneath it.

d. That the ossicles came to overlap in a complex but consistent pattern.

2. Recent work seeks to discover a possible role for collagenous patterns in the perilimbic connective tissue in determining the overlap pattern. The most recent findings include the following.

a. Extra papillae (and therefore extra ossicles) appear in the embryonic conjunctiva in one of two locations in the conjunctival papillary ring.

b. A regularity in the direction of overlap of so-called "s" type junctions suggests that the overlap pattern may be dictated by the collagenous grain of the mesenchyme in which the bones develop.

c. Systematic removal of papillae produce gaps in the bony ring which became filled by ingrowth from adjacent ossicles which eventually overlap. Analysis of these experimentally produced overlaps suggests that a minimum of three "fields" of different collagenous grain exist in the region of the sclerotic ring.

Significance to Bio-medical Research and the Program of the Institute: These studies make contributions to our knowledge in two areas. The first deals with the factors which shape, or misshape the vertebrate eye during its embryonic development. The second, which uses the scleral ossicles as an appropriate model, seeks to determine the morphogenetic functions of collagen which is deposited early in embryonic development.

Proposed Course of Project: The general project (ocular morphogenesis) will be continued for sometime to come. There is at least one more year of work planned for the ocular skeleton.

Honors and Awards: Jonas Friedenwald Award of the American Association for Research in Ophthalmology, 23 April 1969.

Publications: None

Serial No. NDS(I)-69 O/EE 1662(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Lateral Specificity in the Neurogenesis of the Optic System of the Chick Embryo

Previous Serial Number: HD-DB2

Principal Investigator: Jane L. Coulombre, B.S.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: We sought to establish whether or not the axons of the retinal ganglion cells are specified as to side (right or left eye), and the extent to which this is part of the basis for the establishment of highly regular patterns of decussation during embryonic development. It was planned to ask when such specificity appears during development, whether or not it was species-specific and whether it arose intrinsically or was dictated by tissues which surround the eye.

Methods Employed: Microsurgical techniques developed in this Section were used to transplant the eyes of chick embryos from one side of the head to the other early in development. The independent variables were: host age, donor age, donor species, and the sidedness of the graft. The dependent variables dealt with the pathways taken by axons growing out of the transplanted eye, and whether they reached the right or the left tectum. Silver impregnation techniques were used to determine the course taken by the axons leaving the graft.

Major Findings: Preliminary results indicate that when either right or left eyes from three day donors are transplanted to the right orbital bed of

three-day hosts, all their axons grew to the contralateral tectum. Thus specification for laterality had not occurred in the retinal ganglion cells by three days of incubation.

Significance to Bio-medical Research and the Program of the Institute: In the development of neuronal circuity, connections are established not only among the several levels of the nervous system, but also from one side of the body to another in a complex but orderly pattern of cross-connections. We had hoped to shed light on when these cross-connections are established, whether the order in which they develop is obligatory or whether each step is independent, what properties of outgrowing axons and of the primitive nervous system through which they grow guide the fibers to establish appropriate connections, and to answer a number of related questions. Answers to these questions are essential to: a) any understanding of how information encoded at the genetic level achieves expression in the developed nervous system, and b) determining when during development and why one or another pathway or nucleus may be at hazard to insult introduced from without.

Proposed Course of Project: After an exhaustive appraisal of the technical difficulties encountered in transplanting eyes older than three-days of incubation the project was terminated on 1 September 1968. The decision to terminate the project at this stage was based on the development of new opportunities with greater immediate promise.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 O/EE 1663(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Modulation of the Anterior Epithelium of the Cornea

Previous Serial Number: NONE

Principal Investigator: Jane L. Coulombre, B.S.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.6
Professional:	0.9
Other:	0.7

Project Description:

Objectives: In the chick embryo, and presumably in the embryos of other vertebrates, the anterior epithelium of the cornea becomes differentiated very early in development. This project seeks to test the reversibility of this differentiation by attempting to reprogram the epithelium for differentiation in another direction.

Methods Employed: The lenses were removed from the right eyes of a group of chick embryos at five days of incubation. In one subgroup these lenses were replaced by pre-dermal mesenchyme from the metatarsal regions of thirteen day donor embryos. When the host animals had achieved fourteen days of incubation the graft-containing eye was examined grossly, fixed and analyzed histologically.

Major Findings: 1. By five days of incubation the anterior epithelium of the cornea has differentiated on the basis of several criteria. 2. At this time the anterior epithelium will redifferentiate to form feathers under the influence of pre-dermis with feather forming potency. 3. The five-day anterior epithelium of the cornea forms scales in response to contact with pre-dermis with scale forming potency. 4. Both of these tissue interactions require proximity between the interacting tissues. Under the conditions of the experiment this is brought about by breakdown of the primitive stroma which lines

the anterior epithelium. 5. The tissue interaction has a low species specificity. Thus the anterior epithelium will form feathers in response to mouse pre-dermis with hair-forming potencies.

Significance to Bio-medical Research and the Program of the Institute: This analysis increases our understanding of the nature of the interactions among embryonic tissues during early development. Specifically it opens the way to determine the time it takes the cells of the corneal anterior epithelium to become committed, or to determine whether they ever become fully committed. The degree of fixity or plasticity of differentiated ocular tissues is an important determinant of the extent to which regeneration or reconstruction of eye parts can be brought about.

Proposed Course of Project: The project will be continued for at least another year. There are plans to explore such independent variables as: The age of the anterior corneal epithelium; the age of the donor tissue; the site of origin of the mesenchymal tissue; the species of origin of the donor mesenchyme. A publication of the initial findings has already been submitted in abstract form to "Investigative Ophthalmology." Full publication will follow completion of the work.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 O/EE 1664(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development and Functional Significance of the Centrifugal Fibers in the Chick Visual Pathway

Previous Serial Number: NONE

Principal Investigator: Richard J. Lederman, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: The aim is to study the influence of the optic tectum on the developing centrifugal pathway of the chick visual system and to determine the role of the latter in the development and function of the retina.

Methods Employed: Complete or partial removal of the optic tectum during early development was performed, using suction. High-frequency electrocoagulation has also been used to make small lesions in the midbrain. Routine Nissl and silver staining techniques were used to assess the effects of these lesions.

Major Findings: The source of the centrifugal fibers to the retina is known to be a large collection of cells in the midbrain, the isthmo-optic nucleus. Fibers from the optic tectum are the major, if not the sole, afferents to the isthmo-optic nucleus. Early removal of the optic tectum has been shown, in the course of this research, to have a profound influence on the development of the ipsilateral isthmo-optic nucleus. A rough correlation appears to exist between the amount of tectum remaining and the size of the fully-developed isthmo-optic nucleus. Although a point-to-point projection is known to exist from the former to the latter, it has not been possible, so far, to demonstrate the selective development of one portion of the isthmo-optic nucleus by partial ablation of the tectum.

A series of experiments has recently been initiated to destroy the isthmo-optic nucleus selectively, in hopes of determining the influence of the centrifugal fibers on the retinal amacrine cell development and on visual function. While several technical problems remain, a method for fixating the embryo at a particular orientation in situ, using gentle suction, has been developed.

Significance to Bio-medical Research and the Program of the Institute: An understanding of the role of the centrifugal fibers in the development and function of the visual apparatus will contribute to the elucidation of the more general phenomenon of central control over the input and relay of sensory information more peripherally.

Proposed Course of Project: Further refinements will be made in the technique of localizing and limiting the lesions made to the area of the isthmo-optic nucleus. The effects of these lesions on the parameters mentioned above will be studied.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 O/EE 1665(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effects of Drugs Administered to Chick Embryos on Post-Hatching Behavior

Previous Serial Number: HD-DB4

Principal Investigator: Stephen W. Parker, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: The objective is to determine whether pharmacologic agents (epinephrine, atropine, and chlorpromazine) can modify post-hatching behavior when they are administered to the chick embryo.

Methods Employed: The pharmacologic agents are introduced continuously into the incubating chick egg by a modification of the catheterization and continuous infusion technique previously developed in the Section on Experimental Embryology, and acutely by injection into the yolk sac. The behavioral tests are the following of a flickering light stimulus (James) and the Scholes detour problem.

Major Findings: Epinephrine, atropine, and chlorpromazine, in the doses given, dripped on the chorioallantoic membrane of the chick embryo from day eight or nine of development through day twenty of development, do not significantly modify behavior of chicks in the situations tested.

Significance to Bio-medical Research and the Program of the Institute: Among mammals, including human beings, drugs administered to the pregnant female often reach her embryo or fetus in effective concentrations. A good deal of attention has been given to the production of physical defects in

such cases. Little is known about the extent to which behavioral patterns may be altered by prenatal exposure to pharmacologic agents. This study seeks to find out in what ways behavior may be modified by directly exposing the embryo to various drugs.

Proposed Course of Project: This project will be terminated June 30, 1969 when the principal investigator leaves NIH.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 O/EE 1666(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Lens Fiber Formation In Vitro and In Vivo

Previous Serial Number: HD-DB5

Principal Investigator: Joram Piatigorsky, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.9
Professional:	0.9
Other:	0.0

Project Description:

Objectives: The purpose of the present study is to reveal the pattern of synthesis of macromolecules during elongation of epithelial cells into lens fibers in the chick embryo.

Methods Employed: The methods include tissue culture, histological methods and the conventional biochemistry of nucleic acids and protein using radioactive tracers.

Major Findings: Philpott and Coulombre (Exp. Cell Res. 38: 635, 1965) showed that excised epithelial cells of the lens of six day embryos of chicks elongated to form fiberlike cells when cultured with fetal calf serum. In the absence of serum the epithelial cells retained their original morphology. Daily histological measurements in the present study showed that the rate of elongation was linear for at least a week with a doubling of cell length having occurred by 24 hours.

Experiments with H^3-uridine and C^{14}-valine demonstrated that RNA and protein synthesis are stimulated both in epithelial cells and in fiber cells by fetal calf serum. An elevated rate of protein synthesis continues for one to two hours in epithelial cells and for at least nine hours in fiber cells after inhibition of RNA synthesis with actinomycin D. After nine hours in actinomycin the epithelial cells incorporate valine at approximately half their inital rate.

RNA synthesis increases about two-fold in epithelial and fiber cells in response to fetal calf serum. Phenol extraction and sucrose density-gradient centrifugation revealed that H^3-uridine incorporation into 28S and 18S ribosomal RNA largely accounts for the observed increase in RNA synthesis. Two hour exposures of the cells to H^3-uridine resulted in pronounced labeling of ribosomal precursor 45S RNA in the absence of fetal calf serum and very little labeling of the 45S RNA in the presence of serum. Taken together these facts suggest that 45S RNA conversion to 28S and 18S ribosomal RNA may serve as a regulatory mechanism to limit the rate of 45S RNA synthesis. Synthesis and processing of 45S RNA may, however, be under entirely independent control.

Significance to Bio-medical Research and the Program of the Institute: The results of this study add to our understanding of how, during the course of normal development, cells already determined as lens cells synthesize cell specific proteins. The mechanisms controlling this process underlie the development of an appropriate size, shape and orientation of the lens during embryonic life.

Proposed Course of Project: Dr. Piatigorsky will continue this project. On 1 July 1969 the project will return with him to NICHD.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 O/EE 1667(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of the Embryonic Determination of the Presumptive Lens Ectoderm in the Chick Embryo.

Previous Serial Number: NONE

Principal Investigator: Joram Piatigorsky, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: Other workers have already established that the lens ectoderm becomes determined (committed to its fate as lens tissue) under the influence of the presumptive neural retina at the tip of the optic vesicle. This determination occurs early in development, at stage 11, in the chick embryo. It is also well established that, later in development, the differentiating neural retina influences lens epithelial cells to form lens fibers. It is the objective of this study to determine whether these two influences are the same or different.

Methods Employed: The methods include: enzymatic and microsurgical dissection of embryonic tissues, transplantation of grafts into the eye of the five-day old chick embryo and histological procedures.

Major Findings: This project was recently started, and the techniques for carrying it out are still being developed. The following are the accomplishments to date:

1. The trypsin dissection technique has been adapted to the removal of surface sheets of ectoderm from early embryos.
2. It has been determined that the zone between the tip of the optic vesicle and the presumptive lens ectoderm is trypsin resistant.
3. A procedure has been developed for isolating the presumptive lens ectoderm prior to its contact with the optic vesicle, and for implanting such isolates into the lentectomized eye of the five day chick embryo.

Significance to Bio-medical Research and the Program of the Institute: This study seeks to determine whether the retinal influence which leads to lens cell determination is similar to or different from the retinal influence which leads to lens fiber differentiation. The results will have a bearing on the more general problem of the similarities and differences between induction and other types of tissue interaction. They should also deepen our understanding of normal ocular morphogenesis and the mechanisms which underlie it.

Proposed Course of Project: Work will continue to achieve the objectives of the study. The project will be moved to the Developmental Biology Branch, NICHD on July 1, 1969 when Dr. Piatigorsky rejoins that Institute.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 O/EE 1668(c)
1. Ophthalmology Branch
2. Section on Experimental Embryology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Early Morphogenesis of the Cornea of the Chick Embryo.

Previous Serial Number: HD-DB6

Principal Investigator: Robert L. Trelstad, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description:

Objectives: During early corneal development the corneal epithelium excretes a collagenous matrix into the subepithelial space. This acellular matrix determines the organization of the adult corneal collagen. This study seeks to define the factors which control the deposition of the embryonic collagenous matrix.

Methods Employed: Light and electron microscopy. Autoradiography.

Major Findings: The early acellular matrix is comprised of layers of collagen fibrils organized in an orthogonal pattern. During the early stage of deposition of this subepithelial collagen the epithelial cells which produce it undergo a change in shape. The cells become elongated in an axis which is in register with one axis of the underlying collagen fibrils. Dense intracytoplasmic vacuoles which possibly contain the pre-excreted collagen are also elongated in shape and aligned along the long axis of the cell. During the later stages of deposition of the collagen fibrils the epithelial cells lose their elongated shape. The collagen fibrils continue, however, to be deposited beneath the epithelium in an orthogonal pattern.

Significance to Bio-medical Research and the Program of the Institute: During embryogenesis structures of different shapes and functions are developed. A number of different factors probably operate simultaneously to influence the ultimate shape of a structure. The factors which operate during the morphogenesis of the cornea may apply to other developing tissues as well.

The cornea is particularly useful for such studies because of its relatively simple yet precise construction and its accessibility for experimental manipulation.

Proposed Course of Project: The microscopic evaluation of the cornea is nearly complete. The results are being prepared for publication. This project will be completed by 30 June 1969.

Honors and Awards: None

Publications: None

Serial No. NDB(I)-63 O/OC 1022(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Methotrexate Therapy of Selected Patients with Uveitis

Previous Serial Number: SAME

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: This project is being continued to explore the role of antimetabolites in the treatment of refractory ocular inflammatory conditions.

Methods Employed:

I. Drug Therapy: (1) Methotrexate is given intravenously twice weekly at 25 mgm/M^2 body surface area. (2) Cyclophosphamide is administered weekly at 1 gm/M^2 body surface area. (3) Imuran - 3.0 mgm/kilo/day in 4 equally divided doses.

II. Laboratory Studies: (1) Standard radial immuno-diffusion technique was used to quantitate the three classes of immunoglobulins (IgG, IgA, IgM) in the sera of treated patients. The levels of immunoglobulins were compared prior to and after cessation of immunosuppressive therapy. (2) Primary immune

response was examined by administering subcutaneously 0.1 mgm of Vi (Vi), tularemia vaccine (Tu), and pneumococcus (Pn) antigens. Antigen was given one week after the start of therapy. Antibody titres were determined before and at two and four weeks after treatment had begun. (3) Blood for determination of iso- and febrile agglutinins was obtained to ascertain the effect of immunosuppressive therapy on secondary antibody formation. (4) After immunosuppression had begun, a sensitizing dose of 2,000 µg DNCB (2, 4, Dinitrochlorobenzene) was applied to the skin of the upper arm. Testing doses of 50 µg and 100 µg were reapplied at two weeks to evaluate the influence on the induction of delayed hypersensitivity. (5) Positive Mantoux tests obtained on admission were considered as evidence of delayed hypersensitivity. Patients with positive reactions were rechallenged with the same antigen during the on-therapy period four to six weeks after chemotherapy had begun. (6) The effect of systemically used antimetabolites on an induced local inflammatory response was studied by employing the skin-window technique. Each patient served as his own pretreatment control. The procedure was repeated after two weeks of therapy and 24 hours after the injection of chemotherapy.

Major Findings: During the past year, four corneal homograft reaction cases were treated with antimetabolites because of complete and partial rejection in 3 cases and in the fourth patient with some signs of an impending graft failure. All surgery was performed elsewhere and had failed conventional medical therapy postoperatively 10 days to 3 months before referral to the National Institutes of Health.

Evidence of decreased inflammation was observed in three patients treated with antimetabolites. Notably, pericorneal injection became less and this was accompanied by decrease in photophobia. In two of the cases, the improvement was more pronounced with clearing and reversal of edema in the homograft. The patient with the least amount of inflammation was maintained on Imuran for 12 months with complete reversal of all signs of rejection. The operated eye has remained perfectly clear with normal vision since discontinuation of all medication for the past 3 months. In the second of the latter two patients, discontinuation of therapy was elected because of drugs toxicity.

Some degree in suppression of corneal neovascularization was seen in each case during the on-therapy period of treatment. However, on cessation of antimetabolites, 3/4 patients had rapid vascular invasion of their homografts followed by edema and opacification.

As noted previously in our uveitis studies, varying degrees of altered immune responses were obtained with antimetabolites but consistent correlation between clinical results and the extent of immunosuppression could not be established.

The effectiveness of immunosuppressive therapy in corneal homograft rejection must be interpreted with caution at the present stage of our evaluation. Firstly, 4 cases are too few to be meaningful. Moreover, any comprehensive studies of proportion are made difficult by the number of variables inherent in any transplantation of tissues, i.e., various existing diseases leading to transplantation are not the same,differences in surgical technics, etc.

Nevertheless, better case selection in the future as well as a programmed treatment schedule will undoubtedly provide constructive guidelines in the proper use of antimetabolites in corneal homograft reactions.

Significance to Bio-medical Research and the Program of the Institute: This project is combined within the Branch's program on uveitis.

Proposed Course of Project: The project is being continued.

Honors and Awards: None

Publications:

Wong, V.G.: The use of immunosuppressive therapy in ocular inflammatory diseases. Arch. Ophthal. (In Press)

Serial No. NDS(I)-67 O/OC 1436(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Toxic Effects of Drugs on the Retina.

Previous Serial Number: SAME

Principal Investigators: Eliot Berson, M.D.
Peter Gouras, M.D.

Other Investigators: None

This project has been terminated.

Serial No. NDS(I)-68 O/OC 1534(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Retinal and Uveal Changes Produced Experimentally by Inoculation of Retina Antigen

Previous Serial Number: SAME

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia A. Grimes, B.S.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description

Objectives: To attempt to produce in rats the ocular allergic responses to retinal antigen previously observed in monkeys.

Methods Employed: 10 Lewis-Fisher rats were inoculated with a suspension of rat retina (20 mg/animal) emulsified in complete Freund's adjuvant. An additional group of 10 animals was injected with the adjuvant alone. The rats were observed regularly with the slit lamp and ophthalmoscope and at the end of the experiment the eyes were examined histologically.

Major Findings: No inflammatory changes of uvea or retina were observed clinically during a 6 weeks observation period.

At the end of this interval the animals were given a second injection of the retinal antigen. No ocular abnormalities developed in the subsequent 8 weeks and all animals were killed. Histological examination was negative.

Significance to Bio-medical Research and the Program of the Institute: Autoimmune mechanisms might be operative in the development of intraocular inflammatory diseases. The resemblance of the experimentally induced autoimmune retinopathies to vascular retinopathies in man may suggest therapeutic approaches in the future.

Proposed Course of Project: This project is complete.

Honors and Awards: None

Publications:

Lerner, E. M., Stone, S. H., Myers, R. E., and von Sallmann, L.: Autoimmune chorioretinitis in rhesus monkeys. Science 162: 561-562, 1968.

von Sallmann, L., Myers, R. E., Stone, S. H.: Retinal and uveal inflammation in monkeys following inoculation with homologous retinal antigen. Arch. Ophthal. 81: 374-382, 1969.

Serial No. NDS(I)-68 O/OC 1535(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of Cellular Proliferation in Ocular Tissues

Previous Serial Number: SAME

Principal Investigators: Ludwig von Sallmann, M.D.
Patricia Grimes, B.A.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2.0
Professional:	1.2
Other:	0.8

Project Description:

Objectives: 1) To continue efforts to define the factors affecting proliferation and differentiation of the epithelial cells of the rat lens maintained in vitro. 2) To examine the characteristics of cultured trout lens epithelial cells in view of the extensive epithelial proliferation and lens capsule formation previously observed in experimentally induced cataracts of fish. 3) To study the effects of the cytotoxic agent, dibromomannitol, on cell proliferation in the lens epithelium to compare its mode of action with that of myleran, a structurally related cataractogenic compound. Both drugs are used in the treatment of chronic myelogenous leukemia. 4) To investigate the course of cataract development in rats during streptozotocin-induced diabetes.

Methods Employed: Intact rat lenses were cultured under conditions described previously.

Cultures of trout lens epithelial cells were established from explants of lens capsule with attached epithelium. The explants were spread on cover-slips and maintained in Leighton tubes in medium 199 with 20% fetal calf serum at room temperature. Each week between 1 and 13 weeks after culture was started, two cover-slip preparations were fixed in formalin and stained with periodic acid-Schiff-hematoxylin. One of the preparations was incubated with diastase prior to staining.

The effects of dibromomannitol on H^3-thymidine incorporation, mitosis, and cell death in the lens epithelium were examined after a single injection of the drug and during chronic administration. Methods used for the evaluation of the proliferative activity of the lens epithelium have been described previously.

Rats injected with streptozotocin were examined regularly with the slit lamp to follow the course of cataract development. Non-fasting blood glucose levels were measured daily during the first week after injection and at weekly intervals subsequently.

Major Findings: 1) By more rigorous control of gassing and osmolality of the medium, the culture system has been stabilized to the extent that all explanted lenses survive in vitro. Lenses incubated in the basic medium produce lactic acid at a constant rate during culture periods as long as 7 days, indicating that a relatively active metabolism is maintained. Further study of the effect of culture on the lens epithelium has demonstrated that even in the basic medium there is a stimulation of cell division in the normally non-proliferating central region. Increased mitotic activity appears after 48 to 72 hours of culture. The addition of insulin to the medium leads to a greater burst of mitotic activity which occurs approximately 24 hours earlier. Hydrocortisone had no effect on this system.

2) Cultures of epithelial cells of the trout lens were readily established and were observed to produce an extracellular, PAS-positive, fibrous substance assumed to be lens capsule material.

The lens capsule represents an epithelial basement membrane produced by the lens cells. Although epithelial cells from the lenses of chicks and several mammalian species have been successfully cultured in the past, the possible formation of capsule substance in vitro has not been investigated.

Outgrowth of the epithelium from the edge of the explant was present in 6 day old cultures and became more extensive with further lapse of time. The cells were epithelioid in type and generally formed a regular mosaic pattern. Small, PAS-positive, diastase-resistant granules were embedded in the cytoplasm of many cells. After 2 weeks of culture strands of material with the same staining properties appeared extracellularly. They did not follow the boundaries of cells but crossed over the cell mosaic in an irregular fashion. Cell preparations cultured for 5 weeks or longer showed, in addition to the thick strands, a fine meshwork of PAS-positive material throughout the areas where cells formed an epithelioid sheet. Frequently, clumps of PAS-positive material were seen within the cytoplasm. It could not be determined whether these intercellular inclusions represented continuous formation of capsule material or phagocytic activity of the epithelial cells.

3) Dibromomannitol (DBM) has a profound effect on the proliferative capacity of the lens epithelim in rats. A single injection of 1 gm/kg body weight causes a rapid increase in both H^3-thymidine labeled cells and mitoses during the first week after drug administration. Despite the apparent stimulation of proliferative activity, signs of cell injury and death were seen by 2 days and became progressively more extensive. The initial increase of H^3-labeled cells and mitotic activity occurs prior to the appearance of cell loss and probably represents a direct effect of the drug on these phases of the cell cycle rather than a compensatory response to the injury. Whether it indicates a true stimulation of proliferation or a prolongation of the synthesizing period and the mitotic process has not been determined.

When DBM is fed to rats at a level of 2 gms/kg diet the lens epithelium shows progressive injury and, by 4 to 8 weeks, severe depletion of the population with total loss of the germinative zone. This drastic loss of cells occurs in the presence of a maintained increase in the number of mitoses.

Anterior cortical opacities have been observed in these animals after 4 weeks of drug administration. DBM, therefore, resembles myleran in its ability to damage the lens epithelium and to induce cataract under experimental conditions, but differs from myleran in the manner by which it interferes with the cell cycle.

4) Streptozotocin, a new diabetogenic agent, is reported to be very effective and less toxic than alloxan. In view of the extensive investigation of experimental diabetic cataracts induced by alloxan, the effects of streptozotocin diabetes of the lens were studied. All of 18 rats injected with streptozotocin (65 mg/kg i.v.) showed a persistent elevation of non-fasting blood glucose levels from 24 hours to 8 weeks after administration of the drug. Sugar concentrations were above 300 mg/100 ml for the duration of the experiment. No animals died from the effects of the drug. Incipient lens opacities were first seen at 10 days in about half of the experimental animals. All of the rats had lens changes at 14 days and by 4 weeks showed advanced anterior cortical opacities. The cataracts were similar in clinical appearance and course to alloxan-induced cataracts in rats of the same age. Histological examination of lenses at various stages of cataract formation has not as yet been completed.

Significance to Bio-medical Research and the Program of the Institute: The lens epithelium is a relatively simple cell system particularly well suited to study the life cycle of the cell. Knowledge of the population dynamics which is obtained in this tissue under physiological and pathologic conditions can be related to the problem of cellular proliferation and differentiation in general.

Proposed Course of the Project: Further study will be made of the factors which lead to failure of differentiation in the lens epithelium in culture and the induction of the abnormal proliferative phase. Investigation of the effect of the dibromomannitol will be continued in an effort to establish the mode of action of the drug at the cellular level. The evaluation of cataract formation in streptozotocin-induced diabetes will be completed.

Honors and Awards: None

Publications:

von Sallmann, L., Grimes, P., and Albert, D.: Histogenesis of the lens capsule in tissue culture. Am. J. Ophthal. (In Press), 1969.

Serial No. NDS(I)-68 O/OC 1537(c)
1. Ophthalmology Branch
2. Office of the Chief

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effects of Intrauterine Surgical Procedures on the Development of the Primate Eye

Previous Serial Number: SAME

Principal Investigators: Ludwig von Sallmann, M.D.
Ronald Myers, M.D., Ph.D.

Other Investigators: None

This project has been terminated until further experimental material becomes available.

Serial No. NDS(I)-68 O/OC 1539(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Episcleral Venous Pressure and Glaucoma

Previous Serial Number: SAME

Principal Investigators: Thomas F. Minas, M.D.
Steven M. Podos, M.D.
Frank J. Macri, Ph.D

Other Investigators: Ralph D. Gunkel, O.D.

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description:

Objectives: To investigate the episcleral venous pressure in normal human eyes, eyes with primary open angle glaucoma, and eyes with other forms of glaucoma and to measure pseudofacility and episcleral arterial pressures.

Methods Employed: The episcleral venous pressure was measured with a new instrument developed for this purpose. A modified force-displacement transducer with a lucite cone applanating tip was mounted on a slit lamp biomicroscope and applied to the desired vessel, one could then determine the pressure necessary to arrest blood flow. Measurements were made on a variety of human eyes, normal and glaucomatous.

Major Findings: Selected cases of glaucoma were studied to determine if elevation of episcleral venous pressure was present.

No new cases of glaucoma secondary to elevated episcleral venous pressure were discovered.

A patient previously found to have unilateral idiopathic elevated episcleral venous pressure has been followed and the effect of antiglaucoma drugs studied. No change of her episcleral venous pressure has been observed, and no conclusions have been reached concerning effectiveness of therapy.

Pseudofacility of aqueous humor outflow was studied in normal and glaucomatous eyes. The results compare favorably with those of other investigators. An attempt has been made to study the effect of antiglaucoma drugs on pseudofacility, but no conclusion can be made from the results so far.

Episcleral arterial pressure was measured in patients with unilaterally reduced ophthalmic artery pressure as determined by ophthalmodynamometry. The measurement of episcleral arterial pressure did not yield reproducible results or appear to have any advantage over ophthalmodynamometry.

Significance to Bio-medical Research and the Program of the Institute: The measurement of episcleral venous pressure is of importance in further understanding human aqueous humor dynamics. The diagnosis and therapy of some forms of glaucoma related to episcleral venous pressure elevation may be facilitated by elucidation of their pathogenesis.

Proposed Course of Project: The episcleral venous pressure of the eyes with a variety of ocular diseases is being studied. The project will be terminated at the end of this fiscal year.

Honors and Awards: None

Publications:

Podos, S. M., Minas, T. F., and Macri, F. J.: A new instrument to measure episcleral venous pressure: comparison of normal eyes and eyes with primary open angle glaucoma. Arch. Ophthal. 80: 202-208, 1968.

Minas, T. F. and Podos, S. M.: Familial glaucoma associated with elevated episcleral venous pressure. Arch. Ophthal. 80: 209-213, 1968.

Serial No. NDS(I)-68 O/OC 1540(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cystinosis

Previous Serial Number: SAME

Principal Investigator: Vernon G. Wong, M.D.

Other Investigators: Jerry A. Schneider, M.D.
Toichiro Kuwabara, M.D.
Joseph Schulman, M.D.

Cooperating Unit: Laboratory of Human Genetics
NIAMD

Man Years

Total	0.5
Professional:	0.5
Other:	0.0

Project Description

1. Objectives: Biochemical comparisons of the benign and nephropathic forms of cystinosis.

Methods Employed: A Beckman Spinco amino acid analyzer was used in the assay of cystine in leucocytes, skin fibroblasts and plasma. For subcellular fractionation, leukocytes and fibroblasts were lysed by sonication and separated by differential centrifugation in 0.25 M sucrose.

Major Findings: The plasma concentration of cystine tended to be lower in both the nephropathic (9 patients) and benign (3 patients) forms of cystinosis than in normal controls.

The free cystine content of leukocytes and fibroblasts from the nephropathic patients was about 100 times higher than from normals whereas the hetero-zygotes (carriers) of this disorder averaged 5-6 times that of normal. On the other hand, the free cystine content in the benign form of this condition was found to be 30 to 50 times higher than normal. The increased cystine in the two forms of the disease was localized in a subcellular granular fraction separable by centrifugation at 27,000 g for 10 minutes. This intracellular deposit of free cystine is unavailable for sustaining normal metabolism since neither cell type was viable in a cystine free medium.

By this method, the determination of free cystine identification of the carriers (hetero-zygotes) of nephropathic cystinosis was achieved for the first time. The availability of a biochemical marker has provided new dimensions in genetic counseling of this potentially fatal disease.

2. Objectives: Development of a simple technic for the biochemical diagnosis of cystinosis. Conjunctival crystals from affected patients have been identified as L-cystine and have been found to be identical to those crystals found in other tissues. The diagnosis of this metabolically inherited disease is based on the identification of these crystalline deposits.

Method Employed: Bulbar conjunctival tissue is excised under local anesthesia and the tissue is weighed before extraction of cystine in 0.5 ml of 0.1N HCl. The acid extract is then assayed by column chromatography. Biopsy material may be stored indefinitely in absolute alcohol without affecting the accuracy of the assay.

Major Findings: We have been able to clearly distinguish all cystinotics (7 patients) from normals in the cases so far tested. The cystine contents of biopsies from normals were always below the level of detection possible by this method i.e., less than 0.05-0.10 mμmoles 1/2 cystine. In cystinotics the cystine contents were never less than 5 and usually greater than 30 mμmoles 1/2 cystine/mgm wet weight of tissue.

The biopsy is easily performed and is without complications. Simplicity and negligible morbidity of this technic has proven to be superior to bone marrow aspiration and/or WBC determination of cystine for diagnosis.

3. Objectives: Our recent studies have shown that the accumulation of cystine crystals may be related to a high intracellular concentration of the amino acid rather than a high plasma cystine concentration. The biochemical evidence suggested that the increased cystine is compartmentalized in a subcellular organelle. For this reason, bulbar conjunctiva was obtained for electronmicroscopic examination from one sibship of each clinical form of cystinosis.

Method Employed: Biopsies were immediately fixed in buffered 4% glutaraldehyde at pH 7.6. Tissues were embedded in epoxy resin for thin section examination.

Major Findings: The subepithelial connective tissue contained an increased number of fibroblasts and histiocytes in which polymorphic crystalline structures were distributed intracellularly. Most of these crystalline spaces were delineated marginally by intact double membranes. Small crystalline aggregates were confined within osmophilic dense bodies having the characteristics of lysosomes. It appears that the observed large crystals represent growth products initiated from the membrane limited small crystals.

The study suggests that there is a marked morphologic similarity in the benign and nephropathic forms of cystinosis at the cellular level. The possibility exists that this disorder may arise from a defect of cystine metabolism in the organelles in which the cystine crystals are stored. Whether or not the two forms represent genetic variations in the severity of expression of the same underlying primary metabolic defect remains to be demonstrated.

Significance to Bio-medical Research and the Program of the Institute: This is a continual effort in attempts to delineate, characterize and to elucidate the pathogenesis of the metabolically inherited disorder of cystinosis.

Proposed Course of Project: The project is being continued.

Honor and Awards: None

Publications:

Schneider, J.A., Wong, V.G., Bradley, K., and Seegmiller, J.E.: Biochemical comparisons of the adult and childhood forms of cystinosis. New Eng. J. Med. 279: 1253-1257, 1968

Serial No. NDS(I)-69 O/OC 1669(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Lens Changes and Chemotherapy

Previous Serial Number: NONE

Principal Investigators: Steven M. Podos, M.D.

Other Investigators: George P. Canellos, M.D.

Cooperating Units: Medicine Branch, NCI

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: To investigate the role of chemotherapeutic drugs in the production of human lens changes.

Methods Employed: Myleran production of experimental toxic cataract in rats has been documented in the literature. The eyes of 42 patients with chronic granulocytic leukemia (CGL) were studied without any knowledge of the patients therapeutic regimen.

Major Findings: Three patients had posterior subcapsular lens opacities, 10 had irregularity and polychromatism in the posterior subcapsular zone with or without vacuoles and 29 were free of such changes. The presence of lens changes correlated with the known duration of disease and duration of therapy with the two major chemotherapeutic agents utilized, Myleran and dibromomannitol. In age-matched groups of patients with CGL,

the mean duration of therapy with dibromomannitol and/or Myleran was 30.0 ± 4.9 months for those with lens changes as compared to 8.1 ± 2.0 months for those without lens changes.

Significance: This clinical study possibly implicates Myleran in the production of posterior subcapsular cataracts. This potential, although not striking, side effect of certain chemotherapeutic agents may be important to the clinician. The role of the underlying disease is uncertain with respect to the cataract production.

Proposed Course of Project: Other patients are being studied and followed to further document the initial findings. An attempt is being made to acquire lenses removed from patients treated with such agents for laboratory study. The project will be terminated as of July 1969.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 O/OC 1670(c)
1. Ophthalmology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ophthalmic Manifestations of the Wiskott-Aldrich Syndrome

Previous Serial Number: NONE

Principal Investigators: Steven M. Podos, M.D.
Richard B. Einaugler, M.D.
Daniel M. Albert, M.D.

Other Investigators: R. Michael Blaese, M.D.

Cooperating Units: Metabolism Branch, NCI

Man Years

Total:	0.2
Professional:	0.2
Other:	0.0

Project Description

Objectives: To review the general medical literature and three patients seen with the Wiskott-Aldrich syndrome with respect to ocular disease.

Major Findings: Eighteen out of 80 cases of the Wiskott-Aldrich syndrome documented in the literature had ocular complications of bleeding or infection. Blepharoconjunctivitis associated with molluscum contagiosum, and herpes simplex keratitis, isolated and/or accompanied by disseminated mucocutaneous herpes simplex virus infection, were found in our cases.

Significance: The high prevalence of ocular findings should alert Ophthalmologists to further study patients with this syndrome with special emphasis on immunologic mechanisms and infection.

Proposed Course of Project: Completed

Honors and Awards: None

Publications: None

HOW TO USE
THESE SEPARATORS

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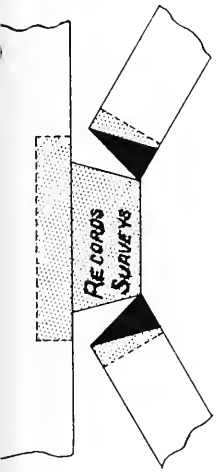

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1968 through June 30, 1969
Branch of Electroencephalography and Clinical Neurophysiology
National Institute of Neurological Diseases and Stroke

Cosimo Ajmone Marsan, M.D., Chief

Summary of Program Activity

1. Clinical-Diagnostic Service

This Branch has continued to provide a clinical-diagnostic service for all the patients in the Clinical Center (both in- and out-patients) referred for EEG examination, either as part of their routine diagnostic work-up or as part of investigative projects originating outside the Branch (or Institute). This form of activity represents a considerable portion of the over-all activity of the Branch, monopolizing more than half of the time of the technical and secretarial staff and over one-third of the time of the professional personnel.

From the time the last report was prepared (April 1, 1968) to that of the present report (April 1, 1969) a total of 1391 examinations have been carried out in the EEG Laboratory on patients referred from the various Institutes, with the following distribution:

INSTITUTE	NO.	%
NINDS	823	59.2
NCI	208	14.9
NIMH	112	8.1
NIAMD	96	6.9
NHI	87	6.3
NIAID	36	2.6
NICHD	17	1.2
NIDR, EHS	12	0.8
	1,391	100.0

As in the past the largest number of referrals have been from our Institute (especially from the Branch of Neurological Surgery), although over 40% of the examinations were requested by other Institutes, with the NCI accounting for 17/month. In the total of the examinations performed for NINDS are included special studies such as those obtained by means of surface and depth electrodes surgically implanted (and kept in place for variable periods of time) in subjects affected by seizure disorders

and by various forms of involuntary movements. A total of 26 such records were obtained in 7 patients. In addition to the routine (scalp) examinations and to those obtained with chronically implanted leads, 9 electrocorticograms were obtained acutely in the Operating Room to assist the neurosurgeon in the outline of the cortical areas to be excised for treatment of seizures.

The functioning of this service is now considered to be relatively satisfactory inspite of the reduced number in technical personnel. It should be pointed out, however, that the service would be severely affected by a further reduction in the technical staff.

2. Research Activity

Of the seven projects outlined in the last annual report, three have been completed and the results have already appeared in three recently published articles. Particularly worth mentioning among these is the work on "Re-analysis of the antidromic cortical response. II. In the contribution of cell discharges and PSPs to the evoked potentials". This paper was awarded the first prize in the Hans Berger Essay contest sponsored by the American Electroencephalographic Society.

Four other projects represent a continuation of those which had been initiated in the past fiscal year. Of these, two are in the field of clinical, and two in the field of experimental epilepsy. One of the clinical projects deals with the problem of presence and absence of typical "epileptiform" abnormalities in the EEG tracings of patients which are unquestionably affected by seizure disorders. In such patients, it is not uncommon to find "negative" or even normal EEG records, either occasionally or - though less frequently - through the course of repeated examinations. In this study an attempt is made to evaluate the potential role of a large number of factors in the determination of the EEG pattern. More specifically, the aim of this project is to find out why and in which situations the EEG manifestations of a seizure disorder are absent. For this purpose, 300 patients with a definite diagnosis of epilepsy and in which at least three (and often up to 12) EEG records had been obtained, were selected at random from the EEG files. On the basis of the EEG findings the patients were then subdivided into three main groups: a) with 100% of their records "positive", b) with 100% of their records "negative", and c) with various percentages of "negative" records. The following

factors are subsequently analyzed in relation to each member of these three groups: age, sex, seizure type and classification, presence or absence of grand mal convulsions, etiology, family history, frequency of seizures, time interval between seizure and EEG, medication, etc. The processing of the data and the various correlations (carried out with the cooperation of the Computer and Data Processing Branch of DCRT) has taken more time than it was originally anticipated. The study should, however, be completed within the next few months.

The other clinical project also deals with the EEG patterns of epileptics and, specifically, with the topographical distribution and spread of the "focal" paroxysmal events which are recorded in the interictal periods. In spite of the term, these events are seldom truly focal but tend to appear over more or less wide areas of the scalp. Furthermore, even when a discharge clearly predominates over a given region, its localization is not always constant and, in most cases, the discharge itself tends to appear over (or be projected to) other nearby or distant regions. The location of these 'secondary' foci is often dependent upon that of the main, primary event. This complex situation is additionally complicated by volume conductor phenomena. In order to investigate these problems, the electrical activity picked up by a large number of scalp electrodes is recorded on magnetic tape, and the electrographic patterns are digitalized and analyzed with the aid of a LINC computer. An initial program has been provisionally developed for the recognition of the epileptiform discharges which are considered to originate from the main "focus". Fifty to 100 of such discharges are then averaged and the scalp distribution of this averaged potential is evaluated at various intervals of time following its onset. Data from 33 subjects are currently available on magnetic tape but modifications in the recognition program as well as the most useful form of display are still under study and the project will probably continue for some time before any practical results become available.

In the experimental field one of the projects carried on from the previous year deals with a microelectrode analysis of the epileptogenic action of conjugated estrogen. After topical cortical application this agent has been reported to have the property of inducing characteristic alterations of the surface electrocorticogram; i.e., a pattern that is morphologically reminiscent of that observed in patients

affected by "petit mal" epilepsy. The latter has up till recently defied all attempts to reproduce it experimentally in a convincing way. Inasmuch as there is now a great deal of information on the cellular events within an epileptogenic focus induced by a variety of chemical or physical agents (i.e. in the experimental paradigm of cortical focal epileptogenic lesions in man), it was felt that this experimental situation might provide analogous information and permit interesting comparisons between the two forms of seizure activity. The study is still incomplete, but the results obtained up to date, suggest that no substantial difference exists between the cellular behaviors in the two experimental situations. In fact the microelectrode tracings obtained after premarine application are hardly distinguishable from those obtained after application of strychnine, penicillin, local cooling, etc. The project should be completed in the next few months.

Another, similar study has re-examined the mechanism of action of strychnine in the production of its well known epileptic effects on cortical neurons. Whereas it is true that we possess much information on the membrane behavior of neurons so affected, the intimate or more basic mechanism that might lead to such a behavior is still obscure and/or controversial. In this project (continuation of an analogous, though slightly different project of the previous year), an attempt was made to determine whether "epileptiform" activity can be induced in a single mammalian cortical neuron. Toward this aim double, parallel micropipettes were developed with a distance of less than 50 micra between the two tips and with different tip diameters. With this technique it has been possible to impale a cortical neuron with one electrode tip and then eject iontophoretically strychnine from the other, extracellular, pipette. Although the difficulties inherent to such a technique have greatly limited the amount of satisfactory results, the rare successful experiments have shown that even with this discrete and circumscribed application, strychnine may profoundly affect (in a reversible way) evoked PSPs, increasing amplitude and duration of the excitatory potentials and slightly decreasing the inhibitory ones. These effects would seem to be primarily pre-synaptic, in close proximity to the synaptic terminals. This project has been provisionally terminated due to the transfer of the main investigator to another laboratory. A very similar project, however, was recently started by another investigator. In this project the same

technique is being applied to analyze the epileptogenic action of penicillin. By iontophoretic application of this antibiotic to one cortical neuron and by the simultaneous intracellular monitoring of its activity it should be possible to learn whether the action of this agent is similar to that of strychnine and, hopefully, to determine the still unsettled question of whether its epileptogenic effects are due primarily to an enhancement of excitatory influences or to the elimination of inhibitory ones. Technical problems as well as frequent interruptions and a relocation in connection with the moving of part of the Institute staff to the new building have, up to now, prevented obtaining a sufficient amount of useful data to draw any definite conclusions. It is, however, expected that this project be completed in the first part of the next fiscal year.

Another of the new experimental projects is also directed toward the general problem of epilepsy but its primary purpose is to investigate the distant effects of an epileptic process. The project represents an attempt to analyze, both at the neuronal population and at the single cell level, the interaction between various forms of "normal" activity in regions which are not directly involved in the epileptic process on the one hand, and the typical epileptiform activity on the other. More specifically, it investigates the effects of "pathological" nervous impulses originating from an experimentally induced epileptic focus, upon the spontaneous or evoked activities of distant neurons which are synaptically related to those affected by the epileptogenic agent. A number of such effects were found to occur but these were variable and rather complex, and cannot be summarized here. The problem is of interest not only because of its potential relation with the still unknown, analogous situation in human epilepsy but also in regard to the physiopathogenetic mechanisms that might be involved in the establishment of the so-called "mirror" foci. The experimental part of this project is completed and the analyzed data should be ready for publication in the very near future.

The new project on the "Co-existence of focal and bilateral diffuse paroxysmal discharges in epileptics" has been completed and the results have been reported in an article recently submitted for publication. This project deals with the clinico-electrographic correlations in a group of 35 epileptic patients whose EEG records

are characterized by a rather complex pattern of epileptiform abnormalities and whose correct diagnosis and nosologic classification always present difficult problems. In this study the various clinical and laboratory findings in these patients were compared with those derived from a control group of 67 patients with clearly defined and uncomplicated focal epileptiform discharges. The main (statistically significant) differences characterizing the study group were: a) a lower mean age of onset of seizures; b) the presence of a greater number of patients with foci in the frontal regions and of a smaller number of foci in the temporal region; c) a higher incidence of an excessively slow background activity; d) a greater number of cases with non-electrographic evidence of cerebral abnormality extending beyond the temporal lobe in patients with temporal lobe foci. The study also suggests that the localizing value of foci in the frontal regions, when associated with bilaterally synchronous discharges, is questionable. These cases would thus appear to be poor candidates for possible surgical treatment.

Another new project, also in the clinical field of epilepsy, deals with the association of mental and seizure disorders. This project was recently started and no definite results are as yet available. It is generally believed that patients with the so-called temporal lobe (or psychomotor) epilepsy are those in which mental disorders and/or behavior abnormalities are most common. Yet, on the basis of a very preliminary impression derived from data of the literature and from the cases investigated so far, the evidence in support of such a belief appears to be rather meager. This study should be completed in the coming fiscal year.

The last one among the new projects is not, strictly speaking, a research study but is included here inasmuch as it represents a considerable amount of work by the author on a subject which is of primary interest to him and to the Institute program in general. The project consists of two critical and review analyses which were assigned to him, one ("The epileptic neuron. Acute effects of local epileptogenic agents") to be presented at the Symposium on "Brain Mechanisms of the Epilepsy" and to be eventually included as a chapter of the published symposium; the other to be a chapter ("General and specific aspects of the neurophysiology of epilepsy") in the volume on "Epilepsy" in a new "Handbook of

Clinical Neurology". The comprehensive nature of these two studies does not permit their summary here although their general topic should be obvious from their respective titles. The first one has been completed and its appearance in press is expected before the end of the current calendar year. The second one is about to be completed and should be ready for publication in two-three months.

3. Programs considered for the near future

Research activity, main interests and general field of investigation are expected to continue along essentially similar lines.

Serial No. NDS(I)-68 EEG/CN 1541(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cellular mechanisms in experimental epilepsy: iontophoretic application of strychnine to single cortical neurons

Previous Serial Number: Same

Principal Investigator: Donald R. Humphrey

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

Objectives: The major aim of this project was to determine, by very localized applications of strychnine, whether or not it is possible to produce 'epileptiform' activity in a single, mammalian cortical neuron.

Methods Employed: Experiments were performed on the pre- and post-sigmoid gyri of the cerebral cortex of anesthetized cats. A technique was developed for fabrication of parallel micropipettes, with tips fixed a known distance apart (5-50 micra) and with independently controlled tip diameters.

Major findings: With the use of these electrodes, it was possible in a few instances to successfully impale a neuron while strychnine was ejected iontophoretically from the remaining, extracellular barrel. Whereas problems of recording stability have severely restricted the number of 'acceptable' cells (it is necessary to maintain stable recording conditions for 30-50 min.), the preliminary results indicate that:

(i) Contrary to some previous reports in the literature (all of which involved extracellular recording), application of strychnine only in the vicinity of a single neuron and/or its synaptic terminals, may produce profound effects upon evoked post-synaptic potentials (PSPs) within that cell (increases of 100% in the amplitude and duration of EPSPs, slight depression in the amplitude of IPSPs).

(ii) The effects are reversible (and hence repeatable), with PSPs returning to control levels within 6-8 min. after cessation of iontophoresis.

(iii) The results do not appear attributable to any direct drug effect on the (post-synaptic) cell membrane, at least as one could judge by resting potential levels, spike configuration and amplitude, etc. Rather, they suggest that the predominant effects are exerted pre-synaptically. Since simultaneous recordings from the drug barrel indicated that no neuronal activity was produced, or modified, other than that of the cell under investigation, the affected pre-synaptic region would appear to be in close proximity to the synaptic terminal.

Significance to Bio-medical Research and the Program of the Institute: Should these preliminary findings be confirmed by subsequent experimentation, the combined results would have important implications for current concepts of the 'epileptic neuron'. To date, no investigator has been able to detect significant alterations in the electrical membrane properties of neurons rendered 'epileptic' by applications of strychnine (or penicillin). Indeed, because of this failure, and because of the usual techniques for applying the drug (systemic injection, or topical application to a relatively large region of cortex), investigators have been driven to the conclusion that certain (as yet unidentified) 'pacemaker' neurons are those primarily affected by epileptogenic agents, with the observed cells being secondarily (and synaptically) driven by such units. The present results suggest an alternative hypothesis, viz., that (i) almost any neuron may succumb to the effects of an epileptogenic agent (though there may well be threshold differences) and (ii) that is such effects are primarily exerted upon the axon terminals, it would be impossible to detect significant alterations in the electrical properties of the cell with an intrasomatic electrode.

Proposed Course of the Project: Due to the principal investigator's abrupt transfer to another laboratory, this project had to be temporarily interrupted. However, it is hoped that the study will be continued by other researchers, and, if the new duties permit it, eventually by its originator.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 EEG/CN 1542(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Neurophysiological investigations of drug induced epileptic foci

Previous Serial Number: Same

Principal Investigators: Russell W. Hardy, Jr.

Other Investigators: C. Ajmone Marsan

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.0
Other:	0.3

Project Description:

Objectives: The project aims to investigate the characteristics of single unit activity following the topical cortical application of pharmacological epileptogenic agents capable of inducing a 3/sec spike and wave pattern in the surface record.

Methods employed: The epileptiform activity is produced in the cortex of anesthetized cats by topical application of premarin soaked pledgets. Unit activity is recorded by means of glass microelectrodes. Unit activity is observed during spontaneous epileptiform discharges, and when these discharges are rhythmically "triggered" by stimulation of specific and nonspecific nuclei of the thalamus.

Major findings: It has been possible to produce "spike and wave" activity by the above described technique but, to date, we have not been able to duplicate the 3/sec spike and wave activity characteristic of petit mal epilepsy, even with the help of rhythmical stimulation of various thalamic structures.

Unit activity occurring during the epileptiform waves does not seem to differ substantially from that seen during other forms of drug-induced surface waves. Specifically, the cells within the epileptic focus demonstrate the hypersynchrony of discharge and the paroxysmal depolarization shifts seen in units within penicillin or strychnine foci. There appears to be a temporal correlation of the PDS (and rapid cell discharge) with the surface "spike" and between the cellular IPSP and the surface "wave" although the latter correlation is not as clear as in previously reported examples of spike and waves elicited by non-specific thalamic stimulation.

Significance to Bio-medical Research and Programs of the Institute: The project aims to elucidate the cellular mechanisms responsible for the generation of a special type of epileptiform activity frequently observed in a well known form of human epilepsy.

Proposed Course of the Project: The study should be completed in the next few months.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 EEG/CN 1543(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Factors affecting the occurrence of epileptiform activity in the EEGs of known epileptic patients

Previous Serial Number: Same

Principal Investigator: C. Ajmone Marsan and Lawrence S. Zivin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.7
Other:	0.2

Project Description:

Objectives: Electrographic evidence of a seizure disorder is not always available even in patients which are unquestionably affected by epilepsy. Serial EEG examinations in some of such patients may be positive (i.e., show the presence of abnormal patterns pathognomonic of this disorder) repeatedly and constantly whereas in other patients positive records alternate with negative ones and in still others the records may persistently yield totally negative results. Object of this study is to investigate various factors that may be potentially responsible for presence or absence of typical epileptiform discharges in the record of a proven epileptic patient, with particular emphasis on those cases with consistently negative records.

Methods: This study is carried out in 300 epileptic patients in each of which at least 3 and up to over 10 EEG records have been obtained at various intervals of time in the course of their investigation. On the basis of the EEG

findings the patients are subdivided into three main groups: 1) with 100% "positive" records; 2) with 100% "negative" records; and 3) with various percentages of "negative" records. For each member of these groups the following factors are subsequently analyzed: sex, age, type, localization and classification of seizures, etiology, hereditary factors, frequency of attacks, interval of time between last seizure and EEG, presence or absence of medication, etc.

Major findings: All the information has been collected but the processing of the data and the various correlations are still in course and no definite results are as yet available. A few, preliminary findings would suggest that: a) younger patients tend to have a higher percentage of positive records; b) after interruption of medication the maximum percent of positivity is reached at the 3rd-4th day; c) maximum positivity is reached by the 3rd day following the occurrence of a clinical seizure, and falls off thereafter.

Significance to Bio-medical Research and the Program of the Institute: This study should contribute to the understanding of the EEG features in epileptics and might provide information of both pathogenetic and diagnostic nature in the general field of seizure disorders.

Proposed Course of the Project: This project should be completed in the early part of the next fiscal period.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 EEG/CN 1553(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Topographical distribution and spread of epileptiform interictal "focal" discharges

Previous Serial Number: Same

Principal Investigator: C. Ajmone Marsan and Andrew J. Gabor

Other Investigators: None

Cooperating Units: Laboratory of Technical Development of NINDS and NIMH

Man Years:

Total:	0.8
Professional:	0.5
Other:	0.3

Project Description:

Objectives: To investigate the pattern of distribution and spread of focal isolated paroxysmal discharges as they occur during interictal periods in the EEG tracings of epileptics. Under the assumption that these discharges are the result of propogation of electrical discharges of a neuronal population along anatomically definable pathways, it is hoped that analysis of these patterns will aid in clarifying and better defining the differences or similarities between epileptiform activity arising in different areas of the brain.

Methods employed: The electrical activity of 16 scalp electrodes is recorded on magnetic tape and subsequently digitized and analyzed with aid of a LINC computer. Between 50 and 100 epileptiform discharges are averaged and the distribution over the scalp over a period of 1 second is evaluated.

Major findings: An initial computer program for the

recognition of the epileptiform discharges and subsequent display of the average potential change has been developed. Modifications in the recognition program are now in progress. To date, 33 patients have been studied and appropriate data are available on magnetic tape.

Significance to Bio-medical Research and the Program of the Institute: The project is closely related to one of the major programs of NINDS. It should help in a better understanding of the dynamics of the electrographic manifestations of focal seizures and possibly lead to a more accurate classification and diagnosis of various forms of epilepsy.

Proposed Course of the Project: When the computer program for recognition of the discharges is sufficiently modified, analysis of the data will be carried out. Further collection of data is currently in progress.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 EEG/CN 1671(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on the dissemination of epileptic activity. Cellular mechanisms involved in the interactions between epileptic and normal activity in cat brain

Previous Serial Number: None

Principal Investigator: Robert M. Crowell

Other Investigators: C. Ajmone Marsan

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	0.9
Other:	0.4

Project Description:

Objectives, Methods employed, etc.: The fundamental aim of this study is to elucidate the cellular mechanisms by which epileptic activity in the brain interacts with non-pathologic activity. In order to investigate these interactions, epileptogenic penicillin foci have been produced in pre-cruciate gyrus of the curarized cat, and the activity of "normal" cortex has been monitored by means of gross and microelectrodes inserted in an area homologous and contra-lateral to the focus. Stimulating electrodes in the thalamus of both sides as well as on both cortices have also been employed to elicit epileptic activity or to trigger abnormal discharges as well as to elicit normal responses.

Major findings: In the inter-ictal period, spontaneous or evoked epileptic activity in the penicillin focus is commonly accompanied by brief activation followed by long-lasting inhibition in cortical neurons in the contralateral

cortex. Intra-cellular recordings show that the activation corresponds to summed EPSPs and the inhibition to powerful, long-lasting IPSPs. Stimulation of n. ventralis lateralis (VL) of the thalamus ipsilateral to such a unit often produces cellular firing, but when such VL stimulation is preceeded by a penicillin discharge in the focus, cellular firing is commonly suppressed. Intracellular recordings show that this phenomenon is produced when IPSPs associated with epileptic discharge overcome EPSPs associated with "normal" activity evoked by VL stimulation. Similarly, it has been shown that long-latency, long-lasting IPSPs evoked by VL stimulation ipsilateral to a neuron can suppress EPSPs and spikes associated with epileptic activity which originated contra-laterally to the neuron under study. During ictal events, units contralateral to the penicillin focus may show increased, decreased, or unchanged rates of firing. Spontaneous or evoked high-voltage penicillin waves in the focus are commonly associated with IPSPs and cessation of firing in the contra-lateral cortical neuron during the ictal event. Stimulation of VL ipsilateral to the neuron also produces IPSPs and firing arrest during ictal events. In some cases, however, the influence of the ictal activity is strong enough to blot out every trace of neuronal response to other stimuli. The study has not been completed, but data obtained up till now suggest that many interactions between epileptic and "normal" activities in the cat brain are associated with interactions between excitatory and inhibitory post-synaptic potentials at the neuronal level. The possibility of pre-synaptic inhibition in some cases has not been ruled out.

Significance to Bio-medical Research and the Program of the Institute: This study provides some data relevant to the dissemination of epileptic activity in the brain. Since the observations have been made at the EEG, extracellular, and intracellular levels, it should be possible to use these data to elucidate some aspects of the dissemination of epileptic activity in terms of fundamental neuronal mechanisms.

Proposed Course of the Project: The experimental part of this project is over. Analysis of data, interpretation, and correlation are being carried out at the moment. The project is in its final phase and should be completed in the near future.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 EEG/CN 1672(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Basic mechanisms in the development of penicillin-induced epileptiform activity

Previous Serial Number: None

Principal Investigator: Gregory O. Walsh

Other Investigators: C. Ajmone Marsan

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.6
Other:	0.4

Project Description:

Objectives, Methods employed, etc.: The major objective is to investigate the effects of penicillin iontophoretically applied to a single neuron or its immediate surrounding field in the cortex of cat, using both intra- and extracellular recordings. Penicillin has a well known epileptogenic effect when applied to the neocortex, hippocampus, amygdala and midbrain reticular formation but our knowledge of the intimate cellular mechanisms which are at the basis of such an effect is still fragmentary.

Major findings: To date, most of the time has been spent mastering the electrode technique for intracellular recording and simultaneous nearby iontophoresis. The few extracellular recordings obtained up till now do not permit drawing even preliminary conclusions.

Significance to Bio-medical Research and the Program of the Institute: The cellular mechanisms of epilepsy is one of the Branch's major goals. With this study, which is akin to that

of project 68 EEG/CN 1541, we hope to have more insight into these basic mechanisms.

Proposed Course of the Project: The project will be continued and its completion is expected for the second part of the next fiscal period.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 EEG/CN 1673(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Co-existence of focal and bilateral diffuse paroxysmal discharges in epileptics. Clinical-electrographic study

Previous Serial Number: None

Principal Investigator: Andrew J. Gabor and C. Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.9
Professional:	0.8
Other:	0.1

Project Description:

Objectives, Methods employed, etc.: Whenever isolated or short bursts of focal paroxysmal potentials co-exist with bilaterally synchronous discharges or both types of epileptiform abnormalities are found in sequential closely spaced records of the same epileptic patient, their interpretation and correct diagnostic classification present a difficult problem. The purpose of this study is to assess, in such cases, the significance of the focal discharges or alternatively of the bilaterally synchronous epileptiform activity in terms of the clinical syndrome. A group of 35 patients with the above described complex electrographical aspects were compared with a group of 67 control patients with clearly defined and uncomplicated focal epileptiform discharges. Clinical pathological data derived from the medical records and the electrographic features of the disorder were compared as well. The patients included in the study as well as those of the control group were selected from about 9,000 referrals and a total of 23,000 records. The only requirement for selection of

a patient in the control group was the presence in his record(s) of a unilateral electrographic epileptiform abnormality consistently localized to a restricted area without evidence of bilateral or multifocal involvement.

Major findings: The most significant findings included a relatively high incidence of frontal foci and a relatively low incidence of temporal foci in the study group as compared to the control group. Patients with foci consistent with the clinical seizure pattern, abnormal neurologic examination and contrast radiography had electrographic evidence suggesting a lesion in areas other than the frontal area. The patients of the study group with temporal foci showed evidence of a more widespread abnormality (suprasylvian as well as infrasylvian) than the electrographic focus would suggest. The localizing value of EEG foci in the frontal regions (when such foci are associated with bilaterally synchronous discharges) would appear to be questionable: such foci were generally not supported and in some cases were actually contradicted by the various clinical data.

Significance to Bio-medical Research and the Program of the Institute: The study was undertaken in order to better understand the diagnostic significance of this complex pattern of electrographic abnormality and, hopefully, to select the most suitable form of treatment for those patients in which it appears.

Proposed Course of the Project: This project has been completed. The results have been formally organized and a paper was recently submitted for publication.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 EEG/CN 1674(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Mental disorder and the epilepsies: A review with some observations on correlations between EEG patterns and MMPI scores of epileptics

Previous Serial Number: None

Principal Investigator: Robert J. Mignone

Other Investigators: C. Ajmone Marsan

Cooperating Units: Branch of Neurological Surgery, Section on Clinical Psychology, NINDS

Man Years:

Total:	0.4
Professional:	0.3
Other:	0.1

Project Description:

Objectives, Methods employed, etc.: Purpose of this study is to analyze the occurrence of mental and behavior disorders in epileptics. The literature was reviewed for the past 15 years with regards to the incidence of disturbed behavior in epileptics in their intervals free of seizures. Emphasis was placed on differences between the so-called psychomotor (or temporal lobe) and other epileptics. Attention was also directed to cognitive and personality differences as well. In 109 epileptics in our files and in which MMPI and IQ scores had been obtained, the EEGs are being reviewed for possible correlations between electrographic patterns and localization and MMPI and IQ scores.

Major findings: The older concepts of constitutional deficiencies in intelligence and character cannot be supported for most patients with seizures. When direct effects of focal deficits (motor, sensory, verbal, memory) and of

disruptive seizure discharges are eliminated, one finds intellectual and personality development are subject to the usual multilevel psychobiological dynamics. Furthermore, when sampling bias, relative age distributions, medication effects and seizure classification variations are all considered, one may not find much definite evidence to support differences in personality or mental functioning between psychomotor and other kinds of epilepsies. The retrospective analysis of EEG patterns and MMPI and IQ scores is not sufficiently underway so as to draw any tentative conclusions at this date.

Significance to Bio-medical Research and the Program of the Institute: This is an attempt to define some of the controversies of behavioral differences between epileptics and normals, and between different kinds of epileptics.

Proposed Course of the Project: The project will continue through the first part of the next fiscal period.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 EEG/CN 1675(c)
1. Electroencephalography and Clinical Neurophysiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The "epileptic" neuron

Previous Serial Number: None

Principal Investigator: C. Ajmone Marsan

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description:

Objectives, Methods employed, etc.: This project consists of a description and critical analysis of all the recent data which are related to the development and to the various manifestations of epileptiform activity within the elements of a neuronal aggregate. Such data have been derived mostly from original experimental work carried out in this Branch, and integrated with those from similar investigations in other laboratories. The main purpose of this study is to understand the basic mechanisms leading to what is commonly referred to as the "epileptic neuron"; in addition, an attempt is made to outline the cellular pathophysiology of seizure activity in general as well as the various mechanisms which may be involved in the various types of its manifestations

Major findings: In the first part of this enterprise the main emphasis has been placed on the mode of action and effects of some of the most commonly used epileptogenic agents. Of the changes in neuronal activity which are induced by these agents, two main types ("inter-ictal" and "ictal") are distinguished. These differ from each other not only in their

overall morphology and temporal course but, very probably, also in their underlying mechanisms. This observation leads to the conclusions that the so-called epileptiform activity is not a uniform phenomenon and that it would be unwise to generalize and/or look for a single common mechanism to explain the development of all its manifestations. Thus, for instance, an interference with inhibitory mechanisms may be the crucial factor in the origin of one type of abnormal activity but play only a secondary role (or be non-existent) in the genesis of the other type of activity. Some of the various epileptogenic agents seem to differ in their properties to induce one or the other or both types of activities although it is also possible that, once an epileptogenic process has become established, the transition from "inter-ictal" to "ictal" activity takes place through mechanisms which are relatively independent from the original causal agent. Among these, strychnine has been extensively investigated and in this study an attempt was made to review and summarize all its effects at different levels of both peripheral and central nervous system. The basic pathophysiology of seizures can be usefully analyzed at the level of a single cell or of a local neuronal aggregate but one should not ignore the important role of a number of extrinsic factors. In human epilepsy and, experimentally, whenever an epileptogenic process develops in an individual with intact CNS, the existence of numerous anatomical-functional connections between the various cerebral structures provides the substrate for a complex interplay of facilitatory and inhibitory influences to be exerted upon the process itself. The final effects will depend upon both the specific structure(s) from which such influences originate and the location site of the epileptogenic process. This and other aspects of the problem are discussed in detail in the second part of this project.

Significance to Bio-medical Research and the Program of the Institute: This project represents an attempt to formalize our current knowledge on the pathophysiological mechanisms which are at the basis of the epileptic manifestations. This field is the main interest of this Branch and of the Institute.

Proposed Course of the Project: The first part of this work has been completed. The material was presented at a recent symposium and is currently in press. The second part should be completed before the end of this calendar year and will form the subject of a special chapter in a new Handbook of Neurology.

Honors and Awards: None

Publications: Ajmone Marsan, C.: Acute effects of local epileptogenic agents. In Jasper, et al (Eds.) "Symposium on Brain Mechanisms of the Epilepsies". Little, Brown Co., Boston, 1969, in press.

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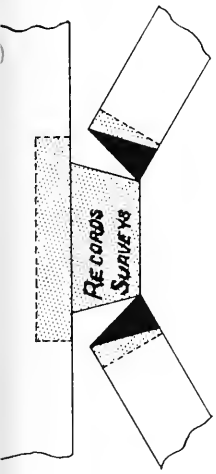

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Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1968 through June 30, 1969
Laboratory of Neuropathology and Neuroanatomical Sciences
National Institute of Neurological Diseases and Stroke

Igor Klatzo, Chief

Introduction

In its second year of existence the LNNS has proceeded further with the endeavor of bringing closer together its "neuropathologically" and "neuroanatomically" oriented sections and investigators. Our aim has been to achieve an overall coordination where data from individual projects can be integrated into a broad front of approaches for the elucidation of some basic neurobiologic problems, especially those with considerable clinical importance.

An area where interdisciplinary collaboration has been fruitfully applied is the problem of the blood-brain barrier which is so important to understanding the normal function and pathological reactions of the brain, and which has been our interest since a number of years. The studies were primarily carried out on the ultrastructural level, using peroxidase as a protein tracer. The following new observations pertaining to the structural localization of the protein permeability barriers were obtained:

1) With regard to the blood-CSF barrier of the choroid plexus it was shown that peroxidase was able to cross the fenestrated endothelium, but not the epithelium of the choroid plexus. The extracellular movement of peroxidase into the ventricle was stopped by tight junctions between the epithelial cells.

2) In experimental brain tumors a significant vesicular (pinocytotic) uptake of the tracer by neoplastic cells was noted. This finding has significance for interpreting the distribution of clinically used radioactive protein tracers, such as RISA, in scanning human brain tumors.

3) In the peripheral nerve (n. sciaticus in the rat), it was demonstrated that the endothelial cells of endoneurial capillaries are joined by tight junctions which exclude peroxidase tracer from the surrounding endoneurium. Similarly, the inner layers of the perineurial sheath are joined by tight junctions which exclude from the endoneurium, protein tracer which has been injected into the nerve or has leaked out of the perineurial blood vessels subsequent to intravenous injection of the tracer. These observations demonstrate the existence of a peripheral nerve-blood barrier and elucidate its structural substrate. The findings have an obvious importance for the interpretation of peripheral nerve diseases.

4) A unique exception to the rule that interendothelial tight junctions constitute the main substrate of the blood-brain barrier to proteins was found in sharks. In these animals, brain capillaries do not show tight junctions between endothelial cells, and a protein tracer readily fills the subendothelial space. On the other hand, the spreading of peroxidase into

the neuropil is prevented at the endothelial border with the glial matrix. These observations may provide important clues about the significance of various morphological components of brain tissue for the passage of proteins. This work on the various aspects of barriers under normal physiological conditions has been conducted through the collaboration of the Sections on Neurocytology and Functional Neuroanatomy.

In the study of pathological aspects of the blood-brain barrier, the main contributions were made by the Section of Comparative Neuropathology. It was discovered that severe effects of ischemia can be modified by perfusing the blood vessels during the cessation of the flow with isotomic or hypertonic oxygen and glucose-free solutions. These observations are important to the problem of temporary ischemic conditions in the human brain. The other interesting finding from these studies is the observation that following a period of ischemia the blood-brain barrier becomes refractive to tracer extravasation which regularly follows the application of barrier-damaging procedures, such as the inducement of hypertension or perfusion with toxic chemical agents. At the moment the significance of these observations is still obscure, but they may harbor important clues for the comprehensive understanding of the barrier pathology and its important role in various brain disorders.

The neural control of cerebral blood vessels undoubtedly may constitute a significant factor in vascular permeability and the behaviour of the blood-brain barrier. The Sections on Functional Neuroanatomy and Neurocytology have been participating in multi-faceted efforts to elucidate various aspects of cerebrovascular innervation. On the ultrastructural level the fine structure of the innervation of cerebral blood vessels was elucidated with respect to the regional control of the cerebral circulation. The findings indicate that nerves running along both extracerebral and intracerebral vessels apparently have effector endings which differ from those of nerves supplying non-vascular smooth muscle. Varicosities of the vascular autonomic fibers, which are filled with synaptic vesicles, approach to within 600 Å of the smooth muscle cells of the vascular walls.

The synaptic vesicles are thought to contain hormonal substances involved in the regulation of vascular tonus and studies in the Section of Neurocytology have constituted an effort to more precisely determine the nature of these substances by combined histochemical and electronmicroscopic observations. It was found that the selectivity of the vital methylene blue stain for autonomic fibers depends greatly on the pH of the staining solution: for example, at pH 6.5-7.0 only cholinergic axons are stained. The observations on the identification of perikaryia and synaptic terminals associated with the presence of norepinephrine, dopamine and 5-hydroxytryptamine at the ultrastructural level will be of great importance in future experimental work involving the identification of nuclei and tracts as well as the effects of drugs on these structures.

In all histological, histochemical and electron microscopic observations the preservation of the tissue is of paramount importance. The Section on Experimental Neuropathology has continued its efforts to improve fixation techniques and it was found that the amount of fixative can be greatly

reduced when the vascular bed is restricted by clamping of the large blood vessels or when the peripheral vasculature is closed off by the use of a vasoconstrictor. Other important research by the Section has been concerned with characteristics of microglia cells. On the basis of these studies an attempt has been made to outline the life history of microglia cells. The detection of microglial cells throughout the brain and spinal cord of all mammals studied suggests that they form an integral part of the central nervous system, and that they are indispensable to the functional capacity of its various elements.

A subject which may represent one of the frontiers of neurophysiology is the question of trophic and hormonal influences of the nervous system. This field has been the main interest of the Section on Experimental Neurology. Investigations of the neural influence on the cholinesterase and ATPase activity of muscle indicate that the nerve specifically regulates the type of myosin synthesized by the muscle fiber. Such a regulation suggests that the nerve may be influencing gene expression. From other studies it appears clear that neither the chemical nature, nor the physiological properties of mammalian extrafusal and intrafusal muscle fibers are immutable, but that they are maintained throughout life by virtue of a still undisclosed neural mechanism. With regard to trophic nerve function as related to sensory systems, an observation demonstrating parallel loss of alkaline phosphatase activity along with the taste buds after denervation has indicated that the enzyme is associated with the taste buds rather than the stratified squamous epithelium. The specific influence of innervation was demonstrated when, after reinnervation by gustatory-type nerves (glossopharyngeal, vagus, chords tympani), regenerated taste buds appeared. However, none appeared after reinnervation by non-gustatory nerves such as the hypoglossus.

Studies on structural interrelationships have been the central research effort of the Section on Functional Neuroanatomy. This work has been primarily related to the morphological elucidation of various sensory systems. In studies on the auditory afferent and efferent systems, two important findings were made. First, the acetylcholinesterase - type fibers and their terminals have been localized more precisely with the electron microscope. Second, the criterion for identifying inhibitory and/or cholinergic endings by other investigators, has been shown by this study to be unreliable.

In another group of studies, anatomical evidence of a fine-caliber system of fibers, which may be concerned with regulation of localized blood-flow to different nuclear levels of the auditory and visual systems, has been revealed with the use of a modified Nauta technique in the cat and the chinchilla. The possibility of a neurogenic mechanism for regulating regional blood flow in the auditory, visual and other systems of the central nervous system is of considerable importance to physiologists studying the problem of localized blood flow of the brain.

Investigations on the fine structure of afferent and efferent nerve endings in the cochlear nucleus have provided much-needed information concerning the ultrastructural alterations that accompany deafferentiation of nerve cells in the brain and have also provided particularly important

information on the identification of different functional types of nerve endings on the basis of ultrastructural dissimilarities.

A preliminary report on the glomerular dendro-dendritic synapses of the olfactory bulb has been published and a full report has been prepared which includes some new data on the origin of these synapses, obtained by combining the Golgi technique with electron microscopy. These observations have already provided a morphological basis for such phenomena as lateral and recurrent inhibition in the olfactory bulb.

Serial No. NDS(I)-68 LNNS/CN 1546
1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Correlation between the size of Extracellular Spaces Assessed by Impedance Measurements and the Various Types of Experimental Edema

Previous Serial Number: Same

Principal Investigator: Choh-luh Li, M.D.

Other Investigators: Barbro Johansson, M.D., and Igor Klatzo, M.D.

Cooperating Units: Surgical Neurology Branch, NINDS

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description:

Objectives: Evaluation of data derived from various experimental models of brain edema has led to classification of this process into two main types, namely - vasogenic and cytotoxic. (Klatzo, I.: Presidential Address. J. Neuropath. & Exp. Neurol. 26, 1-14, 1967). It has been postulated that vasogenic type, related to a primary injury of blood vessels and their leakage, is associated with a dilatation of extracellular spaces, whereas the cytotoxic type, depending on cellular swelling of various morphological components of brain parenchyma, is connected either with reduction or lack of change in extracellular compartment. Although the E.M. observations have widely confirmed the enlargement of extracellular spaces in vasogenic edema, technical aspects of electron microscopy make it difficult to demonstrate changes of extracellular compartment in the cytotoxic type. The present study was undertaken to make such correlations using new and improved methods for measurement of electrical impedance from which it is possible to estimate the size of extracellular compartments.

Methods Employed: The experimental models of brain edema were as follows: 1) edema produced by application of cold metal plate to the exposed cortex of the cat, 2) edema due to embolization produced by intracarotid injection of paraffin material in cats and 3) Triethyl tin edema produced in cats.

Cold lesion edema served as the classic model of vasogenic type of edema. Its various parameters have been extensively studied in this Laboratory. Edema due to embolization is localized primarily in the cerebral cortex and in the other regions of grey matter. Triethyl tin edema has been previously extensively studied in various laboratories. It affects primarily the white matter.

Following inducement of edema in cats, specific resistivity of the cortex and the white matter was determined, action potentials and resting potentials were recorded from single nerve cells in the cerebral cortex and spontaneous electrical activity and transcollosal evoked responses were registered.

For observations on the behaviour of the blood-brain barrier, animals were injected with Evans Blue prior to sacrifice. The brains were fixed by perfusion. The microscopic distribution of the Evans Blue tracer was assessed under the fluorescence microscope. The paraffin embedded material was subjected to various histological stains.

Major Findings: In the cold injury edema the electrophysiological measurements in the affected white matter showed a marked decrease of impedance amounting to a drop by approximately 100% in comparison with the measurements in the normal white matter. Impedance measurements in triethyl tin poisoned cats showed slightly increased values in the grey matter and virtually no change of impedance in the white matter. In cases of edema produced by paraffin emboli there was about 50% increase of impedance in the cortex. These impedance changes after embolization were extremely rapid in onset and preceded any histological changes characteristic for edematous process. The blood-brain barrier changes and histological features of edemas were similar to those previously described. The impedance measurements allowed a fine assessment of the dynamics of the edema process.

Significance to Bio-medical Research and Program of the Institute: This study represents another contribution in a series of investigations concerning cerebral edema, which is of major interest in the Section on Comparative Neuropathology. Electrophysiological measurements of the extracellular compartment in various types of edema were generally in agreement with evaluations by other approaches, specifically those by electron microscopy. Lack of impedance changes in the white matter in cats poisoned with triethyl tin agrees with data by Streicher (J. Neuropath. & Exp. Neurol. 21, 437, 1962), who demonstrated that thiocyanate space does not change in triethyl tin edematous cats. The electrophysiological measurements when properly applied and analyzed may have potential value in clinical evaluation of patients suspected of having brain edema. Sensitivity of these measurements may provide more intimate assessment of dynamics of developing or subsiding brain edema.

Proposed Course of the Project: The experimental work on this project has been completed. The data have to be analysed and prepared for publication.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-68 LNNS/CN 1547
1. Neuropathology & Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effects of Ionizing Radiation on the Elasmobranch Brain

Previous Serial Number: Same

Principal Investigator: Igor Klatzo, M.D.

Other Investigators: Arland L. Carsten, Ph.D.
Milton Brightman, Ph.D., and Yngve Olsson, M.D.

Cooperating Units: Medical Department, Brookhaven National Laboratory, Upton, Long Island, N. Y.

Man Years:

Total:	2.3
Professional:	1.7
Other:	0.6

Project Description:

Objectives: Recent studies have shown the shark brain to exhibit a profound and unusual resistance to changes in vascular permeability and blood-brain barrier following severe local injury (Klatzo, I. and Steinwall, O.: Observations on Cerebrospinal Fluid Pathways and Behaviour of the Blood-Brain Barrier in Sharks. Acta Neuropath. 5, 161-175, 1965). The present investigation is to determine if such a resistance to radiation effects might be also present, and if so, what are the factors causing such a resistance (comparative data is available on mammalian species).

The unique features of cellular morphology such as perivascular arrangement of astrocytes, the ultrastructural characteristics of glia-capillary junctions, distribution of glycogen, etc. create a special interest for studying effects of radiation in Elasmobranchs brain, since it has been shown before that in the mammalian brain the most sensitive changes occur at the glia-blood vessel interphase and involve an abnormal glycogen reaction (Klatzo, et al.: Effects of Alpha Particle Radiation on the Rat Brain Including Vascular Permeability and Glycogen Studies. J. Neuropath. & Exp. Neurol. 20,459-483, 1961). Also, the very different metabolic and oxygen consumption rates of the shark are of particular interest since the relationship of circulation and oxygen tension levels to severity of radiation injury are well established.

Methods Employed: Approximately 50 nurse sharks have been exposed to a well collimated gamma ray beam directed at the cerebellum and underlying portions of the hypothalamus, tegmentum and pons. The radiation source consisting of the cobalt bomb was shipped from the Brookhaven National Laboratory to the Lerner Marine Laboratory, Bimini, Bahamas, and was installed there. The dose range was 1000 - 30,000 r. 25 sharks were irradiated (1000 - 5000 r.) in December 1967 and sacrificed 4 months later. The remaining sharks were subjected to study of acute effects of radiation with dose ranging up to 30,000 r. and were sacrificed within first few days following irradiation. Corresponding in dose radiation was given to cats and those were sacrificed at the comparable time intervals. For the observations on the behaviour of the blood-brain barrier sharks and cats were injected intravenously with the Evans Blue-albumin tracer, usually several hours before the sacrifice. The brains were perfused through the heart by buffered solutions of paraformaldehyde or fixatives designed for the electron microscopy and processed for histological, histochemical and ultrastructural studies.

Major Findings: The tissues from the animals irradiated in Bimini have been shipped to Bethesda and are being currently processed. The gross observations on the behaviour of blood-brain barrier revealed that cats which received 30,000 r. and were sacrificed a few days later showed distinct extravasation of Evans Blue tracer in the irradiated areas, whereas this was not observed in sharks which were subjected to similar dose of radiation and were sacrificed at the corresponding time intervals.

Significance to Bio-medical Research and the Program of the Institute: The Section on Comparative Neuropathology, Laboratory of Neuropathology and Neuroanatomical Sciences, is vitally interested in research on elucidation of some basic pathogenic mechanisms in the nervous system. It is felt that studies on primitive vertebrate and invertebrate brain by confrontation of differences found under various experimental conditions offer a unique opportunity to expand our understanding of intricate neurobiological structure-function relationships. This particular project should contribute to elucidation of various patho-mechanisms operative in the radiation damage of the human brain.

Proposed Course of the Project: The project is roughly in the middle of its execution. The data on acute effects of irradiation will be supplemented with the chronic material from sharks allowed to survive 6 - 12 months after irradiation.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/CN 1681
1. Neuropathology and Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Vulnerability of the blood-brain-barrier (BBB) to protein tracers after temporary cerebral ischemia

Previous Serial Number: None

Principal Investigator: K.-A. Hossmann

Other Investigator: Y. Olsson

Cooperating Units: None

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

Objectives: Previous studies have shown that the BBB to protein tracers remains intact for several hours following cerebral ischemia (Broman et al.). This fact has been interpreted as indicating a high functional resistance of the endothelium (the presumed site of the BBB) to anoxia. However, the concept of the "functional resistance" of the endothelium is in contradiction with the severe structural changes of endothelium observed after a few minutes of cerebral ischemia. The aim of this study is to determine whether the endothelium constitutes the barrier to protein tracers in ischemia, and, if so, to analyze the discrepancy between the functional and morphological preservation of endothelium.

Methods Employed: This study is based on material obtained from 40 cats. Two groups of animals were compared: those in which an ischemic insult was followed by a hemodynamic, chemical, or traumatic injury, and those in which ischemia was not complicated by these "secondary" lesions.

Cerebral ischemia of differing durations was produced in both groups by interrupting the arterial blood supply of the brain without interfering with the venous outflow. The functional impact of the ischemic insult on the cortical neurons was monitored by recording the EEG and the pyramidal response after stimulation of the somato-motor-cortex. The permeability of the blood vessels was assessed by fluorescent and electron microscopy, using

Evans Blue and horseradish peroxidase as protein tracers.

Major Findings: Ischemia uncomplicated by secondary lesions of more than three hours duration did not cause an extravasation of protein tracers. The protein tracers did not reach the basement membrane, indicating that the endothelium remains the actual barrier to those tracers in ischemia. When cats were subjected to secondary lesions, the permeability of the vessels was dependent on the functional impact of the preceeding ischemic insult. No exudations of the tracer were found when ischemia was long enough to suppress the pyramidal response (about 8 minutes), but severe disturbances of the BBB occurred when ischemia was not effective in suppressing this response or as soon as this response recovered.

Significance: The significance of this investigation lies in the observation that a structural damage of the endothelium does not necessarily mean a breakdown of the blood-brain-barrier to proteins. On the contrary, it may produce increased resistance to the effects of different secondary lesions. This observation may provide more clues for the basic understanding of the BBB phenomenon.

Proposed Course of Project: The major part of this investigation has been completed. The data are being analysed in preparation for publication.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/CN 1682

1. Neuropathology and Neuroanatomical Sciences
2. Section on Comparative Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Neurophysiological and morphological studies on temporary cerebral ischemia

Previous Serial Number: None

Principal Investigator: Y. Olsson

Other Investigators: K.-A. Hossmann

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	1.0
Other:	0.6

Project Description:

Objectives: Among the factors which influence the final outcome of a temporary arrest of the cerebral circulation, the post-ischemic impairment of the recirculation ("the non-reflow phenomenon") has been particularly stressed in recent experimental investigations. The impairment of the post-ischemic recirculation is caused by an abnormal swelling of endothelial and glial cells which lead to obstruction of the intracerebral capillaries in the ischemic parts of the brain. The present study was undertaken to analyze the factors involved in the production of the endothelial and glial cell swelling, and to provide means by which this complication could be avoided. For this it was necessary to develop a new method for the production of ischemia and for the evaluation of its functional impact.

Methods Employed: This study is based on material obtained from 60 cats. Temporary cerebral ischemia is produced by interrupting the arterial blood supply to the brain for periods varying from one minute to three hours duration. The cerebral blood flow is studied by surface observations of the pial circulation. The functional impact of ischemia on the brain is evaluated by recording the EEG and the pyramidal response after stimulation of the somato-motor-cortex. This gives us information about the spontaneous neuronal activity and antidromic, as well as orthodromic action potentials of the pyramidal tract cells. The functional sequelae are correlated with light-microscopic and electron-microscopic data.

Major Findings: The impairment of the post-ischemic recirculation can be delayed for at least twenty minutes if ischemia is produced by interrupting the arterial blood supply to the brain without interferring with the venous outflow. However, after post-ischemic intervals longer than 20 minutes, the cerebral blood flow becomes seriously impaired. Yet this impairment of the circulation can be completely prevented if the brain is perfused with isotonic or hypertonic oxygen- and glucose-free solutions during the ischemic period.

Despite the fact that the functional disturbances are the same during the period of ischemia with and without infusion, the recovery of the brain after the ischemic insult differs considerably in the two groups. In the group of cats without infusion, functional recovery is only possible if the ischemia is less than ten minutes. When the impairment of post-ischemic recirculation is prevented by infusion, functional recovery has been observed after ischemic insults of more than twenty-five minutes duration. Severe morphological changes have been observed in the non-infused animals, whereas the infused animals show excellent morphological preservation.

Significance: The prevention of the "non-reflow phenomenon" may have direct clinical importance in conditions with temporary arrest of the cerebral circulation.

Proposed Course of the Project: The major part of this project is completed. It will later be continued by further studies aimed at identifying the pathogenetic factors responsible for the abnormal swelling which underlies the "non-reflow phenomenon".

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 LNNS/ENP 942
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Histochemical study of nerve cells

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To discern the distribution of glycogen-containing neurons.

Methods Employed: Rabbit brains are fixed in situ and prepared according to methods developed in this Section.

Major Findings: There are through the spinal cord and brain stem two types of motor nerve cells which are distinguished by varied histochemical composition.

Significance: The identification of biochemical differences between neurons may give a basis for understanding functional and pathologic differences in reaction.

Proposed Course of Project: Discontinued.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-62 LNNS/ENP 944
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Hematologic control of primates

Previous Serial Number: SAME

Principal Investigator: Mrs. Margaret G. Johnson

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.0
Other:	0.2

Project Description:

Objectives: To control the health conditions of caged primates.

Methods Employed: Different primate species are regularly examined by various hematologic techniques; weight and dietary regimen are controlled.

Major Findings: Many of the primates suffer from severe infections and aberrations of blood composition.

Significance: A careful hematologic examination is required prior to subjecting primates to experiments.

Proposed Course of Project: Regular check of primates and control of infectious condition.

A long term study of the hematologic status in healthy animals.

A long term study of the effect of varied dietary regimen and adjuvants on the hematologic status.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 LNNS/ENP 1065
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Perfection of the perfusion technique for fixation in situ.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.1
Other:	0.6

Project Description:

Objectives: To specify quantities of solutions needed to obtain adequate fixation of the brain and spinal cord.

Methods Employed: Deeply anesthetized animals, in some of which the aorta and subclavian arteries were clamped, were perfused with different quantities of a fixative under varying pressures.

Major Findings: The amounts of fixatives can be greatly reduced when the vascular bed is restricted by clamping of the large blood vessels or the peripheral vasculature is closed off by the use of a vasoconstrictor.

Significance: Since many of the most efficient fixatives are expensive, it is of importance that the quantities needed can be limited to a minimum.

Proposed Course of Project: Partially completed. Manuscript ready for publication.

Honors and Awards: None

Publications:

Cammermeyer, J.: Peripheral vasoconstriction with epinephrine for selective fixation of the central nervous system by perfusion. Acta Neuropath. 11: 368-371, 1968.

Serial No. NDS(I)-63 LNNS/ENP 1066
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Acute degenerative changes in the central nervous system.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: Alden W. Dudley, Jr. (Research Associate, 1963-1965)

Cooperating Units: None

Man Years:

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To compare the changes in material fixed by immersion and by perfusion.

Methods Employed: Experimental microembolization was carried out in various animal species which at different post-operative stages were sacrificed by the perfusion procedure developed in this Section.

Major Findings: A complex of neuronal changes develops in the foci of degeneration.

Significance: To identify the specific acute neuronal changes and to correlate them with those encountered in clinical neuropathology material.

Proposed Course of Project: Microscopic examination is awaiting the histologic preparation of already completed experiments.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-65 LNNS/ENP 1237
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cytologic characteristics of microglial cells

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.5
Professional:	0.5
Other:	1.0

Project Description:

Objectives: To establish the identity of microglial cells under normal and pathologic conditions, and the mode of development of these cells.

Methods Employed: Microglial cells were studied in material fixed by perfusion with Bouin's solution (using the submerged heart method) and in sections stained by a silver carbonate method modified here.

Major Findings: The microglial cells have been found to be inherent elements in the central nervous system, as evidenced by (a) presence in all mammals examined, (b) ubiquitous distribution throughout the central nervous system, (c) occurrence in immature brain tissue, (d) persistence throughout life even in the oldest animals, and (e) development in germfree animals.

According to observations made here, the microglial cell has been found to have a unique mode of development from an extravascular mitotic cell. The intensity of proliferation depends on level of neuron, age of the animal and animal species experimented on. Whereas hematogenous cells seem to be the most important source according to recent studies with autoradiographic methods elsewhere, present observations indicate that microglial cells

possess the ability to proliferate. A mechanism resulting in degeneration of mitotic cells, dysmitosis, enables the tissue to limit proliferation and to prevent over-population of microglial cells. Pericytes do not seem to offer a source for new formation of microglial cells.

Significance: On the basis of this study of the microglial cells, an attempt has been made to outline the life history of these cells. There is considerable controversy concerning the identity and the role of microglial cells. The recognition of microglial cells throughout the brain and spinal cord of all mammals studied suggests that they form an integral part of the central nervous system, and that they are indispensable for the functional capacity of its various elements.

Proposed Course of Project: Terminated. A review of the life history of the microglial cell in historic perspective from the days of Del Rio-Hortega to the present has been readied for publication.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/ENP 1449
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effect of cortisone on the retrograde neuronal changes.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To determine what effect cortisone treatment may have on the acute reactive phase and the subsequent recovery phase of motor neurons subsequent to transection of their axons.

Methods Employed: Cranial motor nerves were cut in different animal species. Serial sections of the brain stem were stained by a combined PAS-gallocyanin method.

Major Findings: Preliminary studies have revealed that the acute peripheral chromatolysis is not influenced by cortisone treatment. The incidence of reactive mitosis is depressed at the five-day stage when, however, the neuronal changes appear to be uninfluenced.

Significance: In the acute stage of retrograde changes, cortisone has been found to have a selective action on the mesodermal elements. The results of this study would form a basis to evaluate the usefulness of cortisone treatment during conditions of nerve regeneration.

Proposed Course of Project: Temporarily discontinued.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/ENP 1450
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Post-operative incidence of mitosis after transection of the facial nerve.

Previous Serial Number: SAME

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.4
Professional:	0.2
Other:	1.2

Project Description:

Objectives: To determine age and species differences in incidence of reactive mitosis.

Methods Employed: Counting of mitotic cells in the facial nucleus of animals fixed by perfusion with the submerged heart method. Both conventional and germ-free animals are utilized. Serial sections of the brain stem are stained by the combined method of PAS and gallocyanin.

Major Findings: After the operation, the sequence of changes in incidence of reactive mitosis varies with age and species. The microglial cells are equally well developed in conventional and germ-free animals.

Significance: When the intensity of mesodermal reaction, as evidenced by incidence of mitosis, to an experimental procedure or noxious agent is being estimated, both the age and species must be taken into account.

Since from earlier studies, it has been shown that the microglial cell is the principal cell evolving from mitosis, it follows that the demand for and the ability to develop microglial cells vary with age and species. The question of whether the microglial cells are primarily concerned with phagocytic activity and reaction to infections in the central nervous system may be solved by a comparison of reactive mitosis in response to a remote trauma in germ-free and conventional animals.

Proposed Course of Project: Results of these studies have been included in a review of microglial cells (Serial No. NDS(I)-65 LNNS/ENP 1237. Terminated.

Honors and Awards: None

Publications:

Cammermeyer, J.: Species differences in acute retrograde neuronal reaction of the facial and hypoglossal nuclei. J. Hirnforsch. 00: 000-000, 1969.

Serial No. NDS(I)-69 LNNS/ENP 1676
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: A comparative study of the area postrema.

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.6
Professional:	0.1
Other:	0.5

Project Description:

Objectives: To determine species differences in the topographic and histologic characteristics of the area postrema.

Methods Employed: Light microscopic techniques.

Major Findings: The size and topography of the area postrema, as well as its permeability to trypan blue, have been found to vary greatly. In some species, such as the pigeon, the area postrema is not developed and in others it is large and Y-shaped or small and massed into a median structure.

Significance: A review of comparative anatomic material may give a clue as to how the area postrema has been transformed during phylogenetic development. On the basis of studies of this kind one may obtain more information as to whether the area postrema serves the same function in all species. Despite considerable work in this field, there is no clear-cut evidence about its functional role.

Proposed Course of Project: Further histologic and experimental work on animals of different phylogenetic development.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/ENP 1677
1. Neuropathology & Neuroanatomical Sciences
2. Section on Experimental Neuropathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Comparative anatomical study of mast cells in the area postrema.

Previous Serial Number: NONE

Principal Investigator: Jan Cammermeyer

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.6
Professional:	0.1
Other:	0.5

Project Description:

Objectives: To determine what role the mast cells in the area postrema may play.

Methods Employed: Histologic methods for identification of mast cells.

Major Findings: Some animals, such as the monkey and dog, contain numerous mast cells in the area postrema while the cat contains very few and other animals none.

Significance: Considerable interest will be attached to these observations since conditions under which mast cells react in the central nervous system and the functional role of the area postrema are not known.

Proposed Course of Project: To study mast cells in serial sections of the brain stem in normal and experimental animals.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-63 LNNS/EN 1054
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: "Trophic" functions of the peripheral nervous system

Previous Serial Number: Same

Principal Investigator: Lloyd Guth

Other Investigators: R. Wayne Albers and Frederick Samaha

Cooperating Units: Laboratory of Neurochemistry, NINDS

Man Years

Total:	2.1
Professional:	0.7
Others:	1.4

Project Description:

Objectives: To investigate the neural influence on the cholinesterase and ATPase activity of muscle.

Methods Employed: (1) Immobilization of the ankle and knee joints in rats (accomplished by internal fixation) was used to produce disuse atrophy of the soleus muscle. The effect of disuse and denervation (produced by sciatic nerve transection) on sole plate and background cholinesterase were compared. (2) Myosin was isolated and purified from fast and slow muscles of the cat. Its properties were studied by gel electrophoresis, and by quantitative and histochemical ATPase techniques. (3) Cross-reinnervation of red and white cat muscles were performed for evaluation of the neural influence on the specific properties of muscle myosin.

Major Findings: (1) Although immobilization and denervation both produce a loss in background ChE, the sole-plate ChE decreases only after denervation, not after immobilization. (2) The myosin from fast muscles had fourfold greater ATPase activity than that of slow muscles, and the enzyme was more alkali stabile and acid labile. This difference in pH sensitivity was also demonstrable histochemically. Most muscles are

composed of two fiber types: type I (low ATPase) fibers predominate in slow muscles and type II (high ATPase) in fast ones. By exposing frozen sections of such muscles to acid or alkali before staining for ATPase, it was found that the type I fibers are acid-stabile and alkali-labile whereas the type II fibers are alkali-stabile and acid-labile. It was found that the differential pH stability of biochemically purified myosin ATPase from fast and slow muscles is correlated with the proportion of type I and type II fibers in these muscles. Electrophoretically distinct "subunits" are released from slow (red) and fast (white) muscle myosin by treatment with p-chloromercuribenzoate. The quantities released from red and white myosin represent 1.6 and 4.0% respectively of the total myosin protein. Removal of nearly all the subunits causes no loss of ATPase activity in red myosin and a two-thirds loss of ATPase activity in white myosin. Disc electrophoresis resolves the "subunits" from white myosin into four bands, A,B,C and D, while those from red myosin are resolved into two bands, B and D. (3) Preliminary histochemical observations on these muscles indicate that, under the influence of the foreign nerve, the muscles synthesize the foreign type of ATPase enzyme.

Significance: (1) These results indicate that the soleplate CHE is under some specific influence of the nervous system; its regulation is apparently unrelated to the degree of use and disuse of the muscle. (2) These studies show that there are qualitative differences between myosin of red and white muscle which can be demonstrated by quantitative biochemical methods and by histochemical techniques and that CMB may be used to study the relationship between the "subunits" and the ATPase activity of myosin. The present demonstration of a differential acid stability of Type I and II fibers explains the reversed staining of these fiber types after EDTA treatment. At the pH of the EDTA solution (4.5), EDTA acts as a buffer, and the loss of staining of the type II fibers results from their acid lability. (3) These results, although preliminary, indicate that the nerve specifically regulates the type of myosin synthesized by the muscle fiber. Such a regulation suggests the possibility that the nerve is influencing gene expression.

Proposed Course of Project: (1) This experiment is completed. (2) Efforts are being made to characterize biochemically the "subunits" released from the myosin by p-chloromercuribenzoate. (3) Studies are under way to determine the exact step in the protein synthetic pathway at which the nerve acts.

Honors and Awards: None

Publications:

Guth, L.: "Trophic" influences of nerve on muscle. Physiol. Rev. 48: 645-687, 1968.

Guth, L.: "Trophic" effects of vertebrate neurons. Neurosciences Res. Prog. Bull. 5: 1-75, 1969.

Serial No. NDS(I)-66 LNNS/EN 1303
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Histo- and biochemical characteristics of muscle sensori - motor components

Previous Serial Number: Same

Principal Investigator: Herbert Yellin

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.7
Professional:	1.0
Others:	0.6

Project Description:

Objectives:

1. To study morphological and histochemical characteristics of sensory receptors in normal and atypical vertebrate muscle.

2. To study, by histochemical methods, the neural regulation of muscle enzymes.

3. To study by histo- and biochemical methods the role of innervation in the manifestation of mammalian skeletal muscle dystrophy.

Methods Employed:

1. Normal, reinnervated, and dually-innervated mammalian skeletal muscle (extrafusal and intrafusal) is being studied by standard histological and histochemical techniques, particularly those demonstrating the disposition and relative levels of glycolytic, oxidative and energy-releasing enzymes (myosin ATPase).

2. Muscles, differentially affected by the dystrophic process are being studied for their myofibrillar, sarcoplasmic

and stromal content (a) under usual circumstances and (b) following reinnervation by alien neurons.

Major Findings:

1. On the basis of their oxidative and glycolytic enzyme activity, some intrafusal muscle fibers appear comparable to, and others different from, the fibers of the extrafusal musculature.

2. Though most muscle fibers of the general somatic musculature exhibit the presence of either the alkali-stabile or acid-stabile forms of myosin ATPase, among specialized muscles (intrafusal and extraocular) a distinctive population of fibers possess both forms of the enzyme.

3. Intrafusal as well as extrafusal muscle fibers appear to exhibit changes in their enzyme-profiles as a result of alien reinnervation.

4. Confirmation of the failure of development of encapsulated proprioceptors in muscles temporarily deprived of their innervation during the myotube stage of development.

5. The histochemical differentiation of muscle fibers in mammalian red and white muscles evolves and is maintained in the absence of the usual proprioceptive influences of the homonymous and juxtaposed muscles.

Significance:

1. The presence in multi-innervated intrafusal (and extraocular) muscle fibers of enzyme profiles different from those of the singularly innervated extrafusal muscle fibers may be attributable to differences in both number and type of innervating neurons.

2. The energy-deriving and energy-releasing enzyme systems and physiological capabilities of intrafusal, as well as extrafusal muscle fibers are under neural regulation. Speed of contraction of muscle can be correlated roughly with muscle fiber enzyme types. It appears quite clear that neither the chemical nature, nor the physiological properties of mammalian extrafusal and intrafusal muscle fibers are immutable, but that they are maintained throughout life by virtue of a still undisclosed neural mechanism.

3. In a variety of primary myopathies there is apparent differential involvement of extrafusal muscle fibers and often

a general sparing of the intrafusal fibers of muscle spindles. If the efferent innervation regulates the contraction-kinetics and energy metabolism of muscle fibers then the following questions arise; (a) Do qualitatively differing efferent innervations protect some muscle fibers from the intrinsic disorders of striated muscle, and (b) are a number of primary muscle diseases dependent upon specific neural influences for their manifestation? If the skeletomotor innervation was a predisposing factor in the relative expression of some primary myopathies, then alteration of the regulatory properties of efferents, or the administration of specific neuromimetic substances might serve to ameliorate certain of these conditions.

4. The usual proprioceptor activity of muscles is not essential to the histochemical differentiation of the extrafusal muscle fibers of homonymous and closely allied muscles. This despite the presumed role of muscle proprioceptors in the modulation of efferent activity, and the suspected role of frequency-of-use of muscle fibers in the establishment of the latter's specific metabolic pathways.

5. The problem of restitution of coordinated motor activity in reinnervated muscle(s) is obviously complicated by the acquisition of various atypical fusimotor-spindle afferent-skeletomotor loops.

Proposed Course of Project: This project will continue as a major portion of the coming year's work.

Honors and Awards: None

Publications:

Eldred, E., Bridgman, C. F., Kano, M., Sasaki, Y., and Yellin, H.: Changes in muscle spindle morphology and discharge with alterations in muscle status. In Yahr, M. D. and Purpura, D. P. (Eds.): Neurophysiological Basis of Normal and Abnormal Motor Activities. Proceedings of the 3rd Symposium of the Parkinson's Disease Information and Research Center. Columbia University. New York, Raven Press, 1967, pp. 35-60.

Yellin, H.: A histochemical study of muscle spindles and their relationship to extrafusal fiber types in the rat. Amer. J. Anat. (In Press) 1969

Serial No. NDS(I)-68 LNNS/EN 1585
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cerebral glycogen metabolism following brain injury

Previous Serial Number: Same

Principal Investigator: Lloyd Guth

Other Investigator: Igor Klatzo

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.3
Others:	0.5

Project Description:

Objectives: Cerebral injury is well known to result in accumulation of glycogen in astrocytes. A quantitative micromethod for glycogen is needed to permit precise investigations into changes in amount, as well as distribution, of glycogen. This project was begun in order to develop a microchemical assay for glycogen that could be applied to paraffin sections for the purpose of doing simultaneous quantitative and histochemical studies.

Methods Employed: A micromethod based on the enzymatic conversion of glycogen to 6-phosphogluconic acid and reduction of TPN to TPNH was used. TPNH formation was measured fluorometrically. The PAS-Dimedon technique for histochemical demonstration of glycogen was also used.

Major Findings: It was found that quantitative recovery of glycogen could be obtained from paraffin sections of brain that had been perfused with 4% phosphate-buffered paraformaldehyde, provided that the fixed brain was not washed in water or dehydrated in low grades of alcohol. The fixation and special dehydration procedures used also gave excellent histochemical demonstration of glycogen. Six hours after a stab wound lesion in the rat cerebrum, a pericapillary

accumulation of glycogen was noted in the vicinity of the lesion. Between 24 and 96 hours postoperatively glycogen deposition was observed in the astrocyte cell bodies and processes. Although these histochemical changes were evident only in the immediate vicinity of the lesion, the quantitative assays revealed increased glycogen throughout the ipsilateral hemisphere. This increase was observed at 6, 24, and 96 hours postoperatively. During the subsequent two weeks the glycogen values returned toward normal.

Significance: These observations emphasize the value of correlated histochemical and quantitative studies on adjacent tissue sections because, if applied independently, the histochemical techniques reveal only gross increases in glycogen and the more sensitive quantitative methods are unable to give any information regarding the distribution of the changes. Furthermore, because glycogen decreases so rapidly following the onset of anoxia, the only previous method for preserving glycogen was by rapid freezing -- a procedure feasible solely in small animals such as the mouse. Studies on the reaction of brain tissue to injury are now possible in larger animals (e.g. monkey and chimpanzee) in which the experimental conditions more nearly correspond to those in man.

Proposed Course of Project: Phyolgenetic and ontogenetic studies on cerebral glycogen after brain injury are contemplated.

Honors and Awards: None

Publications:

Guth, L., and Watson, P. K.: A correlated histochemical and quantitative study of cerebral glycogen after brain injury in the rat. Exper. Neurol. 22: 590-602, 1968.

Serial No. NDS(I)-68 LNNS/EN 1586
1. Neuropathology and Neuroanatomical Sciences
2. Section on Experimental Neurology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Trophic nerve function as related to sensory systems (i.e. taste buds).

Previous Serial Number: Same

Principal Investigator: Andrew A. Zalewski

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.5
Professional:	1.0
Others:	0.5

Project Description:

Objectives: Various phosphatase enzymes are present in the gustatory epithelium of the rat's vallate papilla (innervated by the glossopharyngeal nerves). Adenosine triphosphatase and acid phosphatase are associated with the taste buds, whereas alkaline phosphatase is present only in the superficial epithelium around the buds. The presence of alkaline phosphatase in the superficial epithelium does not indicate whether the enzyme is associated with the taste buds or stratified squamous epithelium wherein the buds are located. If alkaline phosphatase was associated with taste buds the enzyme should disappear after denervation since all taste buds disappear within 10 days after glossopharyngeal nerve transection. On the other hand, if alkaline phosphatase were associated with the squamous epithelium, which undergoes varying degrees of atrophy after denervation, no change or a gradual decrease in the enzyme would be expected.

Alkaline phosphatase is absent, however, from the epithelium of taste buds in the fungiform papillae (innervated by the chorda tympani nerve). Could the presence of alkaline phosphatase in the vallate but not fungiform papillae be due to the difference in innervation? The foliate papilla which has alkaline phosphatase in the epithelium and which is innervated by both the glossopharyngeal and chorda tympani nerve offers a

unique system to answer this question. Selective denervation studies should answer the question as to whether this enzyme regulation is nerve specific.

Since buds in the vallate papilla degenerate after denervation and reappear after nerve regeneration, an investigation was undertaken to determine whether taste bud regeneration was nerve specific or whether other nerves (i.e. general sensory, somatic motor, or different gustatory nerves) could subserve this function.

Finally since testosterone has been demonstrated to cause taste buds to appear in new locations in the vallate papilla, the possible role of testosterone as "the trophic agent" for taste buds was studied.

The present experiments were therefore performed to investigate the role of the nerve in:

(1) regulating enzymes in gustatory epithelium
(2) determining the nerve specificity of enzyme regulation
(3) causing taste bud regeneration
(4) interacting with testosterone to initiate taste bud formation

Methods Employed:

(1) glossopharyngeal nerve transection was performed and alkaline phosphatase activity of the vallate papilla studied histochemically and quantitatively

(2) alkaline phosphatase activity was studied histochemically in the foliate papilla after transection of the glossopharyngeal nerve alone, chorda tympani nerve alone, or after combined glossopharyngeal and chorda tympani nerve transections

(3) taste bud regeneration was studied after reinnervation by the glossopharyngeal nerve (self-reinnervation), hypoglossal nerve (somatic motor), auriculotemporal (general sensory), vagus nerve (gustatory nerve of the buds in the pharynx), or chorda tympani nerve (gustatory nerve of buds on the front of the tongue)

(4) effects of prolonged testosterone administration was studied in adult male rats with an intact innervation of the vallate papilla, with a denervated papilla, and in castrated animals with an intact innervation. The effect of the hormone in reinnervated papillae as described in (3) was also studied.

Major Findings:

(1) After denervation, alkaline phosphatase disappeared

from the vallate papilla. The time sequence of its disappearance coincided with the rate of taste bud loss.

(2) Alkaline phosphatase was still present in the superficial epithelium of those taste buds which remained after glossopharyngeal or chorda tympani nerve transection alone. It was only after both nerves were cut that all taste buds and alkaline phosphatase disappeared.

(3) Regenerated taste buds were found after reinnervation by the glossopharyngeal, vagus, or chorda tympani nerves. None were found after reinnervation by the auriculotemporal or hypoglossal nerves, even when testosterone treatment was combined with these reinnervations. However, when testosterone treatment was combined with gustatory nerve reinnervation (glossopharyngeal, vagus, or chorda tympani) buds were also found in new locations, namely the top of the papilla and area adjacent to it. Buds are not normally found in these locations in the untreated adult male animal.

(4) Testosterone caused buds to appear in new locations (top of the papilla) in normally innervated papilla, gustatory nerve reinnervated papilla, and in castrated animals with a normally innervated papilla. Testosterone treatment of animals with a denervated papilla failed to induce new taste bud formation or to preserve the buds normally present in the papilla.

Significance:

(1) The parallel loss of alkaline phosphatase activity along with the taste buds after denervation indicates that the enzyme is associated with the taste buds rather than the stratified squamous epithelium.

(2) The persistance of alkaline phosphatase in the superficial epithelium of the buds that remained after transecting the glossopharyngeal or chorda tympani nerve alone demonstrated that each nerve alone can regulate alkaline phosphatase activity. The presence of the enzyme in the vallate and foliate papillae, but not in the fungiform papillae must therefore reflect an intrinsic difference of the gustatory epithelia of these regions. The relative independence of alkaline phosphatase of the type of gustatory innervation stresses the importance of continued studies of both nerve and epithelium in gustatory function.

(3) The appearance of taste buds after reinnervation by the glossopharyngeal, vagus, or chorda tympani nerves, but not after reinnervation by the hypoglossal or auriculotemporal nerves indicates that taste bud regeneration is dependent on a property of gustatory nerves that is not shared by motor or general sensory nerves.

The appearance of buds on the top of the papilla and area adjacent to it after combining testosterone treatment and gustatory nerve reinnervations demonstrates that under appropriate conditions additional regions of tongue epithelium can form taste buds.

(4) This result demonstrates that testosterone by itself is not the trophic agent for taste buds. However, testosterone can in some way alter the nerve-epithelia relationship such that taste buds can be formed in new regions.

Proposed Course of Project:

Future studies will attempt to determine:

1) why gustatory nerves can induce taste bud development while general sensory or motor nerves cannot

2) whether epithelium other than oral epithelium can form taste buds (i.e. by transplanting skin epithelium to the tongue).

3) mechanism (s) whereby testosterone causes new taste bud development

Honors and Awards: None

Publications:

Zalewski, A. A.: Changes in phosphatase enzymes following denervation of the vallate papilla of the rat. Exptl. Neurol. 22: 40-51, 1968.

Zalewski, A. A.: Role of nerve and epithelium in the regulation of alkaline phosphatase activity in gustatory papillae. Exptl. Neurol. 23: 18-28, 1969.

Zalewski, A. A.: Neurotrophic-hormonal interaction in the regulation of taste buds in the rat's vallate papilla. J. Neurobiol. 1: (In Press)

Zalewski, A. A.: Combined effects of testosterone and motor, sensory, or gustatory nerve reinnervation on the regeneration of taste buds in the rat. Exptl. Neurol. 23: (In Press)

Serial No. NDS(I)-61 LNNS/NC 808
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The innervation of smooth muscle

Previous Serial Number: Same

Principal Investigator: Keith C. Richardson

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.5
Others:	0.0

Project Description:

Objectives: To study the ultrastructure of autonomic nerve terminals on smooth muscle in collaboration with chemical and pharmacological investigations.

Methods Employed: Standard electron microscopical techniques of fixation and epoxy resin embedding, using neonatal and rat tissues.

Major Findings: The reaction between methylene blue, applied intra-vitally or supra-vitally to tissues containing autonomic nerves, as described in last year's report, has been investigated more intensively. In attempting to standardize the vital staining technique it was found that the selectivity of methylene blue for autonomic axons depends on the pH of the staining solution. At pH 6.5 - 7.0 only cholinergic axons were stained. On lowering the pH to 5.0-5.3 both cholinergic and adrenergic axons took the dye which was readily identified by electronmicroscopy after potassium permanganate fixation. However, it was found that methylene blue uptake is unfortunately somewhat incomplete at the higher pH levels in that some cholinergic axons, identified by their content of agranular vesicles, remain unstained. This limits the usefulness of M.B. vital staining for the identification of cholinergic axons and

the method, as used at present, remains inferior to acetylcholinesterase histochemical techniques, except that it does permit identification of adrenergic axons in the same preparation. The experiments on standardization of M.B. vital staining explain many of the difficulties and discrepancies encountered by light microscopists in using this well known technique.

Significance: As a basis for experimental work on the effects of nerve stimulation and drugs, it is necessary to have reliable indicators of cholinergic and adrenergic axons at the ultra-structural level. While the use of M.B. vital staining combined with permanganate fixation adds a new means of axon identification, the method is limited in usefulness by the incompleteness of cholinergic axon staining.

Proposed Course of Project: Project terminated by resignation and retirement of principal investigator.

Honors and Awards: None

Publications:

Richardson, K. C.: The fine structure of autonomic nerves after vital staining with methylene blue. Anatomical Record (In Press)

Serial No. NDS(I)-66 LNNS/NC 1304

1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Pinocytosis in the brains of dead rats

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

This project is being held in abeyance as the work will have to be reinvestigated with new staining techniques and a new marker (Peroxidase).

Serial No. NDS(I)-67 LNNS/NC 1443
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The intracerebral movement of proteins injected into the blood and cerebrospinal fluid of rodents

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.2
Others:	0.2

Project Description:

Objectives: To examine morphologically the blood-CSF barriers of the choroid plexus to proteins.

Methods Employed: Horseradish peroxidase (6.0 mg) was injected into the femoral vein of mice whose brains were then fixed at different intervals (15 to 20 min.) and examined electron microscopically.

Major Findings: Peroxidase was able to cross the fenestrated endothelium but not the epithelium of the choroid plexus. The protein was incorporated though not transported in appreciable amounts by numerous pinocytotic vesicles. The extracellular movement of peroxidase into the ventricles was stopped by tight junctions between the epithelial cells.

Significance: The structural barriers to the passage of protein from blood into the ventricle reside in the epithelium of the choroid plexus.

Proposed Course of Project: To extend the observations on movement of peroxidase both from the blood and from the CSF and to determine the role of vesicular transport across the

fenestrated endothelium.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/NC 1445

1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The electronmicroscopic identification of adrenergic neurons and nerve terminals in the C.N.S.

Previous Serial Number: Same

Principal Investigator: Keith C. Richardson

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.4
Others:	0.0

Project Description:

Objectives: To identify the ultrastructural characteristics of the perikarya and axon terminals of neurones in the brain of the rat containing norepinephrine or 5-hydroxytryptamine.

Methods Employed: Permanganate fixation, epoxy resin embedding or freeze substitution.

Major Findings: The identification of adrenergic terminals in rat brain has been anticipated by Hokfelt (Zeit. Zellforsch., 79, 1967, 110) who has found that neurones in the locus coeruleus contain small and large granular vesicles in the perikaryon as well as in synaptic terminals. This work confirms the superiority of permanganate fixation for catacholamine-containing vesicles which are not uniformly preserved by glutaraldehyde or osmium tetroxide fixation. However, the identification of neurones containing 5-hydroxytryptamine by electronmicroscopy is still under investigation. Intestinal enterochromaffin cells and blood platelets have been found to contain large granular vesicles indicating that a reaction between 5-HT and permanganate is a possible means of identification of structures known to contain 5-HT. So far similar granular vesicles have not been identified in the nucleus suprachiasmatieus or in regions of the brain stem where fluorescence microscopy demonstrates the presence of 5-HT. The problem is complicated by the low concentration of 5-HT in these neurons as

judged by fluorescence microscopy, and the inevitable loss of the transmitter with fixation. Experiments are in progress using the freeze substitution method of van Harrefeld and Crowell which it is hoped will overcome these difficulties.

Significance: The identification of perikaryia and synaptic terminals in brain associated with the presence of norepinephrine, dopamine and 5-hydroxytrptamine at the ultrastructural level is of great importance in future experimental work involving identification of nuclei and tracts as well as the effects of drugs on these structures. Progress has been slow because of the technical difficulties involved.

Proposed Course of Project: This project will be terminated by resignation and retirement of principal investigator.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/NC 1447

1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: An ultrastructural study of iris innervation after autonomic ganglionectomy in albino rats

Previous Serial Number: Same

Principal Investigator: Duan Roth

Other Investigator: Keith C. Richardson

Cooperating Units: None

This project is terminated and findings have been published.

Publications: Roth, C. D., and Richardson, K. C.: Electron-microscopical studies on axonal degeneration in the rat iris following ganglionectomy. Am. J. Anat. 124: 341-360, 1969.

Serial No. NDS(I)-68 LNNS/NC 1587
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: A blood-brain-barrier to peroxidase in the normal and injured brain of elasmobranchs

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: Thomas Reese, Yngve Olsson and Igor Klatzo

Cooperating Units: None

Man Years

Total:	1.2
Professional:	0.6
Others:	0.6

Project Description

Objectives: To determine the final structural basis for the blood-brain-barrier to peroxidase in the shark.

Methods Employed: Exogenous (horseradish) peroxidase is injected into the caudal vein or cerebral ventricles of nurse sharks and the ultimate distribution of the protein is detected electronmicroscopically by a cytochemical method.

Major Findings: The junctions between adjacent endothelial cells of cerebral capillaries are open in the shark instead of closed, as in mammals and amphibians. Peroxidase is then able to pass between endothelial cells to enter the capillary basement membrane as far as tight junctions between perivascular glial end feet.

Significance: The elasmobranch is, to date exceptional in that the blood-brain-barrier to protein is glial rather than endothelial. The site of these tight junctions thus represents a fundamental difference between mammals and the elasmobranch.

Proposed Course of Project: The findings are being confirmed and have been extended to another elasmobranch (guitar fish) and a teleost (goldfish).

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/NC 1588
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Some intercellular junctions in the vertebrate brain

Previous Serial Number: Same

Principal Investigator: Milton W. Brightman

Other Investigators: Thomas S. Reese

Cooperating Units: None

This project has been completed and findings have been published.

Publications: Brightman, M. W., and Reese, T. S.: Junctions between intimately apposed cell membranes in the vertebrate brain. J. Cell Biol. 40: 648-677, 1969.

Brightman, M. W.: Some conditions necessary for pinocytosis. Proc. EMSA (26th meeting) 142-143, 1968.

Serial No. NDS(I)-69 LNNS/NC 1678
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Distribution of exogenous protein in brain tumors

Previous Serial Number: None

Principal Investigator: Milton W. Brightman

Other Investigators: T. S. Reese, V. Levin, and J. Ausman

Cooperating Units: Office of the Associate Scientific Director for Experimental Therapeutics, NCI

Man Years

Total:	0.5
Professional:	0.3
Others:	0.2

Project Description

Objectives: To determine differences in permeability of the endothelium in neoplasms to peroxidase and the availability of the extracellular spaces (ecs) of the tumors to this protein.

Methods Employed: Pieces of ependymoblastomas are transplanted to mouse brains and, after appropriate periods, peroxidase is administered intravascularly. The brains and their enclosed tumors are fixed by vascular perfusion and examined electronmicroscopically.

Major Findings: The number of cytoplasmic vesicles containing peroxidase is greater than that in normal brains. Peroxidase is able to cross the capillary wall in the tumors and is then able to enter, by vesicular uptake, the tumor cells themselves. The protein is then segregated in vacuoles.

Significance: Unlike the capillaries of adjacent normal brain, those within the tumor appear to be permeable to peroxidase.

Proposed Course of Project: To determine how peroxidase

crosses the endothelium and whether the large ecs of the tumor are thereby filled to account for the large "peroxidase space" estimated by physiological techniques; part of this space may be intracellular (i.e., within damaged cells).

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/NC 1679
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The morphology of photoreceptors in the ventral eye of the horseshoe crab, Limulus polyphemus

Previous Serial Number: None

Principal Investigator: William K. Stell

Other Investigators: Melvyn Ravitz

Cooperating Units: Department of Anatomy, Einstein College of Medicine, Bronx, New York

Man Years

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To study the structure and ultrastructure of photoreceptor cells in the ventral eye of Limulus, in collaboration with the electrophysiological studies of Drs. T. G. Smith, Jr. and George Murray of the Laboratory of Neurophysiology, NINDS.

Methods Employed: Ventral eyes of adult horseshoe crabs were dissected from the living animal. As judged by electrophysiological properties, isolated nerves remain viable for days in cold, unaerated artificial sea water. Therefore they can be subjected to a variety of experimental procedures before preservation for microscopical examination. I have employed the following procedures:

(1) Fixation in isosmotic osmium tetroxide, glutaraldehyde or glutaraldehyde-paraformaldehyde followed by osmium tetroxide, and potassium permanganate.
(2) Fixation in the same agents made hyperosmotic by adding various solutes.
(3) Adaptation to total darkness or bright light before fixation.
(4) Soaking in sea water containing indicators of continuity

with extracellular space, such as horseradish peroxidase, ferritin, and ruthenium red.

(5) Soaking in sea water modified so as to inhibit activity of the "sodium pump", by adding cardiac glycosides (e.g. ouabain, strophanthidin) or excess calcium ions, or by removing all sodium or potassium ions.

Nerves prepared in the above ways were dehydrated, embedded in epoxy resin, and examined in the electron microscope according to standard methods. Some nerves were also sectioned serially at 2.5 μ and the form of individual photoreceptor cells was reconstructed from camera lucida drawings.

Major Findings: The photoreceptor cells of the ventral eye of Limulus are characterized by the same organelles, especially a photoreceptive microvillous border (called the "rhabdomere"), as arthropod photoreceptors which have been described previously. The form of these cells, however, is much more variable, and the distribution of organelles is remarkably more irregular, than in any other invertebrate photoreceptors examined to date.

With all isotonic fixatives, and particularly glutaraldehyde alone followed by osmium tetroxide, close apposition of membranes is observed between the microvilli of the photoreceptor cells and between contiguous cells of all types, including photoreceptor and glia cells. In these close appositions, the external leaflets of the unit membranes are so close together that no space can be discerned between them, and they appear to form a pentalaminar (five-layered) junction. Nevertheless, proteins (horseradish peroxidase) as well as large cations (ruthenium red) can penetrate between the apposed membranes. Hyperosmotic fixation diminishes the incidence of these close appositions, although the junctions of the microvilli are rather resistant to such disruption. Cardiac glycosides, on the contrary, cause the pentalaminar junctions, including those between the microvilli, to disappear completely, even though some swelling of adjacent cells is then observed with isosmotic fixation. Studies comparing these effects to those of exposure to light and to other solutions expected to inhibit the activity of the sodium pump are in progress.

Significance: Electrophysiological studies on the ventral eye of Limulus (Smith et. al., Science, 162: 454-458, 1968) have shown that ions in the bathing fluid appear to have ready access to the photoreceptor cell surface, and that both light and agents which inhibit the sodium pump act similarly to depolarize the cell, reduce its responsiveness to light, and alter its membrane properties. The present morphological studies show that the extracellular channels between photoreceptors and

glial cells are patent to small proteins as well as to ions, even where they appear in fixed material to be occluded by the formation of pentalaminar junctions. This observation casts doubt on the "occluding" property implied for such junctions observed elsewhere. The effect of cardiac glycosides, however, indicates that the forming of such "artifactual" pentalaminar junctions reflects a fundamental property of the cell membranes. By investigating the effects of illumination and other inhibitors of the sodium pump on these junctions, we hope to shed further light on the mode of action of the cardiac glycosides and the relation of the sodium pump to the activity of photoreceptor cells.

Proposed Course of Project: The project is to be completed as outlined above and the findings published within the current calendar year.

Honors and Awards: None

Publications: None

Serial No. NDS(I)- 69 LNNS/NC 1680
1. Neuropathology and Neuroanatomical Sciences
2. Section on Neurocytology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Axonal degeneration in rat cervical sympathetic trunk

Previous Serial Number: None

Principal Investigator: Edward Ganz

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	1.0
Others:	0.0

Project Description:

Objectives: To study the fine-structure and time course of axonal degeneration in rat sympathetic trunk following section and/or superior cervical ganglionectomy.

Methods Employed: Rats are subjected to interruption of the cervical sympathetic trunk by single or multiple section, ligature or superior cervical ganglionectomy. The animals are sacrificed from 18 hrs. to 6 months after operation, the trunks distal to section excised and fixed in a variety of fixatives, and examined with the electronmicroscope. The unoperated side serves as control.

Major Findings: The most dramatic initial changes are seen in the Schwann - cell - axon relationship, with Schwann-cell cytoplasm retracting from the individual axon so as to leave bundles of these unmyelinated fibers situated in shallow scallops on the surface of the Schwann cell. The axons are then seen to decrease in caliber over an extended time course varying from weeks to months.

Significance: This pattern of degeneration of post-ganglionic sympathetic fibers is markedly different from that previously described in the iris, where degeneration is virtually complete after 48 hours.

Proposed Course of Project: To continue to follow the degeneration of axons in the sectioned cervical sympathetic trunk in order to determine their ultimate fate. It would be of considerable interest to investigate these fibers electrophysiologically in order to determine whether they are capable of conducting an impulse.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-60 LNNS/FN 712
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The ascending and descending auditory connections in the primates.

Previous Serial Number: SAME

Principal Investigator: Grant L. Rasmussen

Other Investigators: Prof. Jean Desmedt, Université Libre Bruxelles, Brussels, Belgium

Cooperating Units: Université Libre de Bruxelles, Brussels, Belgium

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: In general, to extend previous studies of the ascending and descending system of lower mammalian subjects to the primate which possesses anatomical features more similar to that of man. In 1965 the specific objective was a study of auditory and related cerebral cortical regions that project corticofugally to the medial geniculate body, inferior colliculus and other areas of the thalamus and midbrain. This project was initiated in the laboratory of Prof. Jean Desmedt of the Université Libre de Bruxelles in 1965 for the purpose of establishing an anatomical basis for follow-up physiological studies on the interaction of the higher set of ascending and descending conducting neurons in the process of hearing.

Methods Employed: Lesions of various sizes and different areas of the superior temporal gyrus and surrounding cortical regions were carefully removed by the suction method. Corticofugal degenerated fibers are being traced in serial sections stained by an axonal degeneration method to lower auditory centers and the relative population of fibers originating from the variously located cortical lesions is evaluated. Thirty cynomolgus monkeys were used in this experiment.

Major Findings: All thirty monkey brains experimented upon in Brussels have been processed histologically but observations are too incomplete to give major findings at this time.

Significance: Anatomical knowledge of the cortical auditory connection in the monkey must be obtained in order to design appropriate physiological experiments for testing the functional role of the descending auditory system.

Proposed Course of Project: This project was inactive during the past year due to full time development in other investigations, particularly, a new project concerning the existence of regional vasomotor circuits regulating blood flow of the central auditory and visual systems. More time will be devoted to this project this year and possibly a collaborative anatomico-physiological project on the primate auditory system with Dr. Desmedt will resume.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-60 LNNS/FN 713
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: A study of the auditory afferent and efferent systems including the existence of regional vasomotor circuits of the central auditory and visual systems.

Previous Serial Number: SAME

Principal Investigator: Grant L. Rasmussen

Other Investigators: Donald McDonald

Cooperating Units: None

Man Years

Total:	1.6
Professional:	1.1
Other:	0.5

Project Description:

Objectives: To continue to reveal unknown anatomical neuronal connections of the afferent and efferent divisions of the auditory system; to gain more information about the anatomical and functional interrelationships of these two systems. The immediate problems under study are:

1. To establish at the synaptic level the interconnections existing between the cochlear nuclei, superior olivary complex, the nuclei of the lateral lemniscus and the inferior colliculus as well as the auditory reflex connections with certain motor nuclei and the reticular formation.

2. To continue study at light microscope level of the morphological relationships of certain aberrant pathways and groups of fibers of the auditory and visual systems which appear to terminate about vessels supplying the nuclear masses of each system. The light microscope study is to be correlated by a separate electron microscopic investigation with Dr. Donald McDonald.

Methods Employed: The newer and more effective techniques are chiefly relied upon for demonstration of axonal preterminal and terminal degeneration in the CNS subsequent to making lesions in appropriate regions. Also the Golgi and the histochemical method of Koelle, which we have modified to advantage, are heavily relied upon to furnish information not otherwise obtainable. The Richardson's silver method for demonstrating normal and degenerate unmyelinated fibers on cerebral vessels is employed as well as histochemical and fluorescence methods for demonstrating cholinergic and adrenergic neuronal elements.

Major Findings: 1. The light microscopic observations on the efferent synaptic endings associated with cells of the cochlear nucleus which were published last year have been extended with the aid of the electron microscope through the collaboration of Donald McDonald of this section.

In this study we have successfully adapted Karnovsky's method of localizing acetylcholinesterase (AChE) in the central nervous system. Two significant findings have resulted. First, the AChE-type fibers and their terminals, previously associated with efferent or feedback neurons (Rasmussen, 1967), have been localized more precisely with the electron microscope. The ultrafine AChE reaction product of Karnovsky's technique is deposited on the limiting membrane of these axons, preterminal fibers, and synaptic terminals. These fibers and terminals persist in the cochlear nucleus after the endings of cochlear nerve afferents have been eliminated. Second, the criterion for identifying inhibitory and/or cholinergic endings by the marked flattening of their synaptic vesicles, which has been proposed by other investigators, has been shown by this study to be unreliable. For example, endings in the principal portion of the nucleus which are associated with AChE have flattened vesicles; however, in the granular layer of this nucleus there is another type of ending which is associated with AChE but has round vesicles. In addition, the terminals of the efferent cochlear bundle, which are known to be cholinergic and to be inhibitory, contain distinctly rounded synaptic vesicles and react for AChE. Thus, at the present time the most reliable method of identifying cholinergic nerve endings is the histochemical technique. The results of this study were presented before the American Association of Anatomists meeting in Boston on April 2, 1969. An abstract of this paper appeared in the Anatomical Record 163: 228, 1969, "Association of acetylcholinesterase with one type of synaptic ending in the cochlear nucleus: an electron microscopic study" (by McDonald and Rasmussen).

2. Anatomical evidence of a fine caliber system of fibers which may be concerned with regulation of localized blood-flow to different nuclear levels of the auditory and visual systems has been revealed with a modified Nauta technique in cat and the chinchilla. The fibers concerned originate and course varying distances in the classical well known ascending and descending auditory and visual pathways before departing external-wards to the glial-pial membrane and to become intimately associated with the penetrating vessels supplying the nuclei of each system. Some penetrate the glial-pial membrane but most fibers turn abruptly in reverse direction short of the glial membrane and accompany the intracerebral arteries and veins. The predisposition of these aberrant coursing fibers for intimate association with intracerebral vessels strongly indicates a vasomotor function. However, it has not been possible thus far to determine with light microscope methods whether or not these fibers actually terminate on the smooth muscle vasculature. Therefore, this important question is also being studied with the aid of techniques applicable to electron microscopy under project LNNS/FN 1589.

Significance: 1. Knowledge of the neuronal relationships existing at the synaptic level between the afferent and efferent systems is basic to an understanding of the neuromechanisms of hearing. Such information is essential for a foundation upon which to design physiological experiments for testing the functional significance particularly of the descending conduction system.

2. The disclosure of a neurogenic mechanism for regulating regional blood flow of the auditory, visual and other systems of the CNS would be important to physiologists sutdying the problem of localized blood flow of the brain.

Proposed Course of Project: 1. Study of the afferent and efferent auditory connections of the chinchilla and cat will be continued.

2. To continue investigation of intracerebral vasomotor neuronal connections with appropriate methods for light microscopy until July 1, 1970.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-65 LNNS/FN 1229
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Fine structure of afferent and efferent nerve endings in the cochlear nucleus of normal and experimental animals.

Previous Serial Number: SAME

Principal Investigator: Thomas Reese

Other Investigators: Grant L. Rasmussen

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To determine the ultrastructural difference between the cochlear nerve afferent endings in different subdivisions of the cochlear nucleus and those of efferent fibers originating from higher auditory centers as previously established by light microscopic studies of G. L. Rasmussen.

To study ultrastructural alterations in the nerve cells deprived of synapses (transynaptic degeneration).

Methods Employed: The electron microscopical studies will be correlated with experimental results obtained from light microscopic studies. The brain is to be perfused-fixed with an appropriate fixative to insure good material for electron microscopic study. The afferent type endings are to be identified by ultrastructural alterations resulting from destruction of a spinal ganglion in chinchilla and the time course of this degenerative process studied. The efferent type of endings are to be identified by ultrastructural alterations following destruction of the superior olive. Comparable areas of the cochlear nucleus of the unoperated side is used as a control.

Major Findings: Studies thus far on the normal anterior ventral nucleus reveal two distinct types of synapses based on size of vesicles. In experimental animals, an electronmicrographic study reveals that the typical calyciform endings of the cochlear nerve possessing the larger vesicles disappear while the remaining unaltered non-afferent synapses on the deafferentated cell possess only the smaller vesicles. A preliminary report appears in the Anatomical Record, vol. 153, p. 408, 1966. During the course of this study it has been appreciated that the afferent endings in the cochlear nucleus undergo degeneration by swelling and lysis rather than by shrinkage (with or without neurofibrillar formation) followed by phagocytosis of the axonal fragment by glia, a sequence of events that had come to be accepted as the only pattern of synaptic degeneration within the central nervous system. Studies on other auditory nuclei are too incomplete to furnish any major findings at this time.

Significance: This study provides much needed information concerning ultrastructural alterations that accompany deafferentation of nerve cells of the brain and particularly the identification of different functional types of nerve endings on the basis of ultrastructural dissimilarities. The discovery of a new type of degeneration in axonal endings separated from their cell bodies will lead to alternative interpretations of some experimental neuroanatomical studies which have depended on recognition of degenerating endings with the electron microscope.

Proposed Course of Project: This project received little attention during the past year and will be continued more actively during this year and will include the synaptology, as revealed with the electron microscope, of other subdivisions of the cochlear nuclei.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-65 LNNS/FN 1231
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The fine structure of the olfactory bulb.

Previous Serial Number: SAME

Principal Investigator: Thomas S. Reese

Other Investigators: Milton W. Brightman

Cooperating Units: None

Man Years

Total:	0.0
Professional:	0.0
Other:	0.0

Project Description:

Objectives: To study the fine structure of the olfactory bulb in the rat.

Methods Employed: Olfactory bulbs were fixed with buffered osmium tetroxide and glutaraldehyde by vascular perfusion. The sections were examined by light and electron microscopy.

Major Findings: A hitherto undescribed synapse, viz, dendro-dendritic, has been found in both the olfactory glomerulus and the external plexiform layer. The origins of both the pre- and post-synaptic components of the dendro-dendritic synapses in the external plexiform have been identified by studying serial electron micrographs. By this identification, in conjunction with concurrent physiological studies, it is shown that the dendro-dendritic synapses serve as a pathway for recurrent and lateral inhibition in the external plexiform layer of the olfactory bulb; this finding is the subject of the report, "Dendrodendritic synaptic pathway for inhibition in the olfactory bulb," Exp. Neurol. 14: 44-56, 1966. The dendro-dendritic synapses in the olfactory glomeruli probably serve quite a different function, which is now being studied. Around the olfactory glomeruli is a system of calyceal envelopments of one small granule neuron by its neighbor, reminiscent of the calyces in the vestibular apparatus.

Significance: Our observations have already provided a morphological basis for such phenomena as lateral and recurrent inhibition in the olfactory bulb. It is anticipated that similar synaptic mechanisms will be found in other sensory systems, particularly in the retina where similar synaptic arrangements have already been reported.

Proposed Course of Project: A preliminary report on the glomerular dendro-dendritic synapses has been published and a full report has been prepared which includes some new data on the origins of the dendro-dendritic synapses obtained by combining the Golgi technique with electron microscopy. The completed report on this work will be sent to the American Journal of Anatomy. It is anticipated that another report based on serial sectioning of synapses in the olfactory bulb will be finished in the coming year, bringing this project to a close.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-67 LNNS/FN 1442
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Fine structural study of the movements of protein within the cerebral meninges, the median eminence and area postrema.

Previous Serial Number: SAME

Principal Investigator: Thomas S. Reese

Other Investigators: Milton W. Brightman

Cooperating Units: None

Man Years

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description:

Objectives: To determine the pathways used by substances passing from the cerebrospinal fluid through the meninges into the parenchyma of the brain and into veins and other tissues surrounding the brain. This approach has now been expanded to include the passage of substances between ventricular and subarachnoid cerebrospinal fluid and specialized areas of the brain such as the median eminence and area postrema.

Methods Employed: The cytochemical method of Karnovsky is used to localize purified horseradish peroxidase after it is injected into the cerebral spinal fluid of living, anesthetized animals. A new peroxidase tracer of smaller molecular size, developed this year, is now being used.

Major Findings: Peroxidase injected into the cerebrospinal fluid of the subarachnoid space in the mouse is able to pass between cells of the pia-arachnoid layer, cross the basement lamina of the brain, and pass between the astrocytic processes forming the limiting surface of the brain to reach the generally open interstitial spaces of the brain. However, peroxidase does not

cross from ventricular cerebrospinal fluid into the median eminence and area postrema. The basis of this selective barrier is a system of "tight" junctions within the specialized epithelial cells lining the ventricular surface over these regions. The use of a smaller peroxidase has allowed it to be visualized crossing the capillary pores in the median eminence and area postrema.

Significance: Since open channels are available for exchange of substances between spinal fluid and brain across its pial-glial surface, this exchange would necessarily have a significant passive and nonselective component. However, any exchange between median eminence or area postrema and ventricular cerebrospinal fluid would be limited by the permeabilities of the covering epithelial cells. This epithelium could then serve as a secondary blood-brain barrier situated at the capillary endothelium. The presence of this "barrier" type of epithelium might also be the reason why neurosecretory hormones released into the median eminence enter the hypophyseal portal system rather than escaping into the cerebrospinal fluid. Our work with smaller peroxidases has also elucidated for the first time the route by which these hormones might cross the capillary epithelium to enter the portal circulation.

Proposed Course of Project: The first finding described was included in a comprehensive article on extracellular space and junctions in the brain by M. W. Brightman and T. S. Reese (LNNS/NC 1444). The localization of barriers to the intercellular movement of peroxidase at the dural-arachnoid membrane has not been completed. The work on the median eminence and area postrema has been reported in a preliminary note (Anat. Rec., 1968) and, it is expected, will be completed and fully reported in the next year.

Honors and Awards: None

Publications:

Brightman, M. W., and T. S. Reese: Junctions between intimately apposed cell membranes in the vertebrate brain. J. Cell Biol. 40: 648-677, 1969.

Serial No. NDS(I)-68 LNNS/FN 1589
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The fine structure of the innervation of cerebral blood vessels, with respect to the regional control of the cerebral circulation.

Previous Serial Number: SAME

Principal Investigator: Donald M. McDonald

Other Investigators: Grant L. Rasmussen

Cooperating Units: None

Man Years

Total:	1.5
Professional:	0.9
Other:	0.6

Project Description:

Objectives: To study the ultrastructural relationship of the vasomotor nerve fibers and their endings to the extra- and intracerebral blood vessels of cat and chinchilla.

To investigate particularly the manner of innervation of the penetrating vessels at the ultrastructural level in order to obtain anatomical evidence for differential regional blood flow of the brain indicated by recent physiological studies.

Methods Employed: Appropriate perfusion fixatives and histochemical methods for light and electronmicroscopical study will be used on normal and experimental animals; to study degenerating nerves and their terminals following lesions in different regions of the auditory and visual systems. Control experiments will also be carried out by eliminating the better known sympathetic and afferent innervation of the non-penetrating cerebral vessels, which will involve complete cervical sympathectomy and the cutting of certain cranial nerves.

Major Findings: The electronmicroscopy findings thus far support the recent observations in Nauta preparations (GLR) that certain superficially coursing fibers originating from all levels of the auditory system direct themselves toward penetrating

vessels supplying auditory nuclei. One region in particular studied thus far is near the merging abducens nerves where fine myelinated nerve fibers originating from the inferior colliculus tend to accumulate about penetrating vessels supplying the superior olivary complex. In normal material a study of the ultrastructure of the surface of the brain in this area has shown in addition to numerous small myelinated fibers, myriads of unmyelinated axons, related intimately to penetrating vessels. Our electronmicroscopic studies thus far have shown that nerves coursing on both extracerebral and intracerebral vessels apparently have effector endings which differ from those of nerves supplying non-vascular smooth muscle, e.g., vas deferens. Varicosities of the vascular autonomic fibers, which are filled with synaptic vesicles, approach to within 600 Å of the smooth muscle cells where they undergo a change in configuration. However, at these sites there is no apparent specialization of the muscle cell membrane.

Our studies to disclose the sources of nerves on cerebral vessels have shown that one population of nerves degenerates rapidly following bilateral cervical sympathectomy, while others remain intact. The majority of the fibers remaining react for acetylcholinesterase in preparations using Karnovsky's histochemical technique for electron microscopy. This indicates that these nerves originate in the parasympathetic nervous system and pass to the vessels via cranial nerves or directly from the brain stem. Presently experiments are being pursued to localize more precisely the source(s) of these fibers.

Significance: Anatomical information revealing a neurogenic vasomotor mechanism for regulation of regional blood-flow in different systems such as auditory and visual would be of immediate importance to physiologists studying the problem of differential cerebral circulation.

Proposed Course of Project: This project under the Principal Investigator is to be concluded by September 1, 1969, at which time Dr. McDonald will leave the Section on Functional Neuroanatomy in order to carry on Graduate School work at University of California, San Francisco, California, and obtain a Ph.D. degree in Neuroscience. The significant findings of this project are to be prepared for publication before departure.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/FN 1683
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ultrastructural basis of the blood-nerve barrier.

Previous Serial Number: NONE

Principal Investigator: Thomas S. Reese

Other Investigators: Yngve Olsson

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.4
Other:	0.3

Project Description:

Objectives: To determine the morphological basis for the exclusion of circulating protein from the parenchyma of peripheral nerve.

Methods Employed: Horseradish peroxidase (M.W. 43,000) was injected into the circulation of living mice and then localized ultrastructurally in the sciatic nerve by means of the cytochemical method of Karnovsky.

Major Findings: The endothelial cells of endoneurial capillaries are joined by tight junctions which exclude peroxidase from the surrounding endoneurium. Likewise, the inner layers of the perineurial sheath are joined by tight junctions which exclude from the endoneurium peroxidase that has been injected into the nerve or has leaked out of the perineurial blood vessels subsequent to intravenous injections of tracer.

Significance: These findings show that the blood-nerve barrier in the mouse has a similar morphological basis to the blood-brain and blood-retinal barriers. Thus, in order for infectious agents or drugs to enter peripheral nerve they must

cross special cellular barriers similar to those protecting the brain. These barriers might also permit the nerve to maintain its own special intracellular environment of various ions independently of fluctuations in the blood levels.

Proposed Course of Project: These findings will be presented this June at the Annual Meeting of the American Association of Neuropathologists and will be later published as a definitive paper.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNNS/FN 1684
1. Neuropathology & Neuroanatomical Sciences
2. Section on Functional Neuroanatomy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Structure and function of close intercellular junctions.

Previous Serial Number: NONE

Principal Investigator: Thomas S. Reese

Other Investigators: Milton W. Brightman

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description:

Objectives: To study the structure and distribution of gap junctions and to show directly with the electronmicroscope their permeability to inter and intracellular tracers.

Methods Employed: Peroxidases of various molecular weights are applied to or injected into cells joined by gap or tight junctions and the peroxidase is subsequently localized with the electronmicroscope. The peroxidases were developed by Dr. Ned Feder, NIAMD, who is supplying them for this study.

Major Findings: None

Significance: This study may show that gap junctions are the type of intercellular contact responsible for the movement of substances from one cell to another as, for example, at electrical synapses between nerve cells. Furthermore, it is hoped that the pathways used for this movement can be elucidated.

Proposed Course of Project: This project will be actively worked on during the next year.

Honors and Awards: None

Publications: None

HOW TO USE
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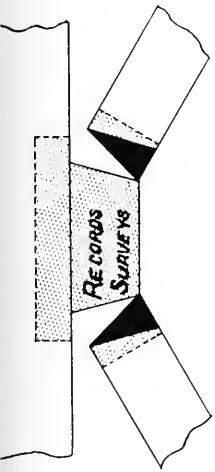

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

Annual Report of the
Laboratory of Neural Control, Intramural Research
National Institute of Neurological Diseases and Stroke

July 1, 1968 through June 30, 1969

Karl Frank, Ph.D., Chief

Laboratory Objectives

The main objectives of this new Laboratory are: (1) the elucidation of fundamental control principles by which information from the nervous system can be used to control external devices, such as prostheses; communications equipment; teleoperators, and computers; (2) the study of methods for control of the nervous system by external stimuli which may be applied either through the sensory receptors or by direct stimulation of the central nervous system; and (3) basic neurophysiological research directed toward the elucidation of principles of control operating in the central nervous system.

Space and Staff

The Laboratory moved into 3,000 square feet of space on the fifth floor of Building 36 in October 1968. Renovation of this space to make it suitable for laboratory operation has been planned but is not scheduled until FY 1970. The staff of the Laboratory now consists of seven professionals, one of whom is a Visiting Scientist, and two secretaries. There are no technicians due to the current employment restrictions. A Russian Exchange Scientist has recently joined the Laboratory for a short-term visit. Two new Research Associates join the Laboratory beginning with the next fiscal year, both with special qualifications in fields related to neural control. A Guest Worker on a Special NIH Fellowship joins the Laboratory for two years in August 1969, and a Visiting Fellow from Australia with special training in motor control mechanisms will join the Laboratory in July 1970. Two summer students may join the Laboratory in 1969.

Collaboration and Contracts

The Laboratory is fortunate in having established a close collaboration with members of the Laboratory of Applied Studies in the Division of Computer Research and Technology. In addition, it is anticipated that the Laboratory will make use of the engineering and scientific expertise of outside laboratories and commercial organizations through the establishment of contracts for carrying out particular directed research programs or for the development of specific techniques and devices. One such contract with the Washington University School of Medicine in

St. Louis, Missouri, is entitled "Development of Instrumentation to Evaluate Sensory and Motor Impairment." A special device has been developed and built for flexing the elbow joint of patients suffering from spasticity and other diseases involving motor impairment, for the purpose of quantitating the degree of deficiency. Preliminary negotiations are under way with the Moss Rehabilitation Hospital in Philadelphia, Pennsylvania, to establish a contract for performing a digital computer pattern recognition analysis of trains of spikes from nerve cells recorded from the motor cortex of monkeys, the data to be obtained by the Laboratory of Neural Control. Another contract possibility being explored is with Case Institute-Western Reserve University for the development of electrodes containing solid state preamplifiers and remote controlled miniaturized micro-positioners for adjusting the position of microelectrodes in the nervous system. Finally, a proposal has been submitted for the establishment of a contract with the Johns Hopkins Laboratory of Applied Physics for the development of a special muscle puller which will permit pre-programmed movement of a joint or a muscle to follow a predetermined displacement or force schedule. One model has already been built and is in use in the Laboratory of Neural Control.

Electrodes

The long-range neural control objectives of the Laboratory are very dependent upon the successful development of multiple parallel pathways for connection with the nervous system. Large macroelectrodes rigidly mounted to the skull are already possible as chronic implants. In addition, microelectrodes similarly mounted have recorded the electrical activity of individual nerve cells in conscious animals for short periods of several days to a week. Such electrodes tend to move with respect to the brain due to its jelly-like mechanical property. Working with a contract engineer in the Section on Technical Development, a technique is being developed for shooting fine flexible electrodes into the brain at high enough velocity to permit penetration without bending to precalculated depths. Such an electrode would move with the brain and so remain fixed in its position relative to particular nerve cells. So far it has been possible to shoot an 0.001" Teflon-coated platinum wire into a gelatin of brain-like consistency to a depth of 10 mm.

Spike Recognition Programs

Fine electrodes frequently record the spike activity of a number of cells simultaneously, whether they be in contact with the muscle fibers of a skeletal muscle, the nerve fibers of motoneurons, or buried amongst the cell bodies of central nervous system neurons. The spike activity of particular cells can be separated from the others on the basis of the amplitude and time course of the spikes. This past year has seen the development of two computer programs designed for such spike recognition.

One developed in collaboration with the DCRT utilizes a hybrid analog-digital computer and has the advantage of allowing optimal sampling time to be determined iteratively by the machine, and thus requires minimal operator intervention. It has the disadvantage, however, that it is off-line and so cannot be directly connected to an on-going experiment.

Another spike recognition program uses the Micro-LINC system. This program separates spikes on the basis of peak amplitude and times for baseline crossings. The system is on-line and so will permit the results of spike recognition and separation to be used in the control of actual experiments. Simpler voltage-window devices for accomplishing the same separations have been developed and built for this Laboratory by the Section on Technical Development. A more sophisticated non-computer spike separation device is under development in the Section on Technical Development.

Cortical Mechanisms of Motor Control

External devices, such as an artificial externally-powered limb prosthesis, can be controlled by the electromyographic potentials recorded from the skin over remaining muscles. A similar control may be possible using electrical signals from the motor cortex. Two investigations are under way in this Laboratory with the long-range goal of obtaining such signals in a usable form. One of these studies utilizes macroelectrodes chronically implanted in particular locations of the motor cortex of cats. Such electrodes show the summed potentials from many nerve cells and processes nearby. It has been possible to demonstrate specific electrical patterns of neural activity which are correlated with the performance of learned motor acts, such as the eye blink. When the same electrodes are used for the stimulation of the cortex, it is found that electrode positions yielding the largest potentials associated with a particular motor act are the positions of lowest threshold for initiating the same motor response by direct stimulation. Comparable stimulus intensities at areas with smaller or absent responses elicit different movements. Adaptive filter techniques have been developed for extracting these very small electrical potential patterns from interfering neural noise. Ultimately, it may be possible to use these neural patterns for the control of external devices.

The other experimental study of voluntary control of electrical activity of the brain involves the use of microelectrodes chronically implanted in the motor cortex of conscious monkeys. Single electrodes capable of recording the spikes from single pyramidal cells have been developed in other laboratories. A technique is under development in this Laboratory to permit as many as five microelectrodes to be independently positioned to record simultaneously from a number of different single cells in the same small volume of nervous tissue. It is anticipated that

the first model of this device will be completed this fiscal year and used to record the trains of spikes from selected pyramidal cells in the monkey's motor cortex associated with specific movements of the arm. In one phase of these experiments, the animal will be rewarded for producing particular patterns of neural activity. Through manipulation of such conditioning, it is hoped to learn the degree of control and resolution possible at the cortical level.

Cortical Pattern Recognition

One of the most important areas of research will be the development of techniques for recognizing the patterns of neural activity associated with particular motor acts. It is planned to analyze multiple spike trains in an attempt to learn the range of variability of patterns of activity from a particular set of electrode positions under normal controlled conditions. From such studies it may be possible to determine the probability of correctly predicting the associated motor act from a given pattern of neural responses. Such a decoding of the signals from the nervous system is necessary for the development of high resolution neural control. It should also lead to important insights into basic neurophysiological mechanisms operating in the nervous system.

Motor Control in Spinal Cord

The largest group in the Laboratory of Neural Control is studying the neurophysiological mechanisms of motor control systems in the spinal cord. This group is investigating the detailed neural circuitry operating in the spinal reflex systems. Motoneurons involved in these reflexes innervate motor units having different mechanical properties. Studies are under way of the relationship between mechanical properties of these motor units and the neural activity of the cells to which they connect. A longer range objective concerns the supraspinal control systems which play upon these spinal motor mechanisms. Conventional microelectrode techniques are used permitting intracellular current injection, and special methods have been developed for recording the mechanical responses of single motor units. One study has just been completed showing the relation of repetitive motoneuron firing to the mechanical twitch properties of motor units. The results show that all motoneurons innervating slow twitch motor units will fire repetitively to injected current steps, while only about half of those innervating fast twitch muscle units fire repetitively.

Part of this research group has transferred to the Laboratory of Neural Control from the former Spinal Cord Section of the Laboratory of Neurophysiology. The results of their work for most of this fiscal year is covered in annual reports from that

Laboratory. Work begun in this Laboratory is thus in the early stages of development. Studies are under way of the tensions produced by single motor units in response to trains of impulses in single motoneurons. Preliminary evidence indicates that interjection of a single extra motoneuron action potential during the course of regular low frequency firing may greatly enhance the tension output from single motor units. Future studies will attempt to elucidate the manner in which whole muscle tension is affected by the asynchronous combination of twitches in single motor units.

The Laboratory of Neural Control has been organized and put into operation with minimal difficulties largely due to the willing cooperation of all concerned. Its objectives are ambitious but appear to have a good chance of realization.

Serial No. NDS(I)-69 LNLC/OC 1686
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Motor Control Systems in the Spinal Cord

Previous Serial Number: NDB(I)-58 LNP/OC-501

Principal Investigator: Robert E. Burke, M.D.

Other Investigators: Pedro N. Rudomin, Ph.D.
Felix E. Zajac, III, Ph.D.
David J. Mishelevich, M.D.
David J. Richman

Cooperating Units: None

Man Years:

Total:	2.1
Professional:	1.8
Other:	0.3

Project Description:

Objectives: The long-range objective of this project is to provide information in two related areas: (1) the detailed neural circuitry and mechanisms operating within reflex systems which include alpha motoneurons on the efferent side, and (2) the interconnection (at the interneuron level) and functional interaction of different reflex systems. Of immediate interest are two main questions. The first of these concerns the mechanical properties of motor units activated individually at frequencies which occur in intact animals. A study has been completed showing the relation of repetitive motoneuron firing to the twitch properties of motor units. The second question concerns the supraspinal control systems which play upon spinal motor mechanisms and which may enable differential activation of fast twitch and slow twitch motor units.

Methods Employed: Anesthetized or unanesthetized, decerebrate cats are used with conventional microelectrode recording techniques permitting intracellular current injection. In addition, special methods have been developed for recording the mechanical responses of single motor units in limb muscles. Digital computer processing has been utilized in analysis of some of the experimental data.

Major Findings: The experimental series in this project are in the early stages of development, and no firm conclusions can be drawn at this time. Preliminary evidence indicates that interposition of a single extra motoneuron action potential during the course of regular low frequency firing may greatly enhance the tension output from single motor units. Studies were made to determine the presence or absence of repetitive firing of spinal cord motoneurons in response to intracellularly injected steps of current. The results show that all motoneurons innervating slow twitch muscle units will fire repetitively to injected current steps, while only about half of those innervating fast twitch muscle units will fire repetitively. Rapid adaptation in some fast twitch motoneurons suggests a mechanism operating at the single motor unit level which may be useful for initiating quick movements.

Significance to Bio-Medical Research and the Program of the Institute: The problem of motor control in the intact animal involves consideration of the functioning of motor units as relatively independent entities usually active in asynchronous patterns. This project is designed to investigate the functional characteristics of motor units individually under a variety of input conditions in order to arrive at a better understanding of how they may behave in the intact animal. Of particular interest are the neural systems which may operate reflexly, or automatically, and the relation which these have to the systems controlling voluntary movement.

Proposed Course of Project: The program of investigation outlined above was begun in March 1969, and will be continued into FY 1970.

Honors and Awards: None

Publications:

Vyklicky, L., Rudomin, P., Zajac, F.E., and Burke, R.E.: Primary afferent depolarization evoked by a painful stimulus. Science, (In press).

Serial No. NDS(I)-69 LNLC/OC 1687
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Techniques for Making Connections with the Nervous System

Previous Serial Number: None

Principal Investigators: Karl Frank, Ph.D.
David J. Mishelevich, M.D.

Other Investigators: Stanley M. Selkow, M.S.[1/]
John Rinzel, B.S.[1/]
James S. Bryan, M.S.[2/]
Charles S. Hazard, M.S.[2/]

[1/]Laboratory of Applied Studies, DCRT
[2/]Section on Technical Development, NIMH/NINDS

Man Years:

Total:	2.1
Professional:	1.8
Other:	0.3

Project Description:

Objectives: Each of the major goals of the Laboratory of Neural Control requires the development of techniques for making chronic connections to the nervous system for the purposes of selective recording and stimulation. Many parallel channels of information are required from individual nerve cells or small groups of cells of similar function. In accomplishing these objectives, it is necessary (1) to develop new types of chronically implantable electrodes, (2) to make connections between such electrodes and recording or stimulating devices outside the body, and (3) to analyze the recordings from such electrodes so as to separate the signals from individual cells.

Methods Employed: A number of approaches to the above objectives are under consideration. A technique is being developed for shooting fine flexible electrodes into the brain at high velocity to predetermined depths. It is hoped that such electrodes will bend and move with the nervous tissue and so remain fixed relative to individual cells whose activity they record.

A contract with Case Western Reserve University is being considered for development of very fine wire electrodes which might be made rigid enough to penetrate the brain by coating them with some soluble, non-toxic substance which would dissolve away after insertion, leaving the wire lead flexible.

Additional developments considered for contracts include electrodes containing a stage of solid state preamplification and a remotely controlled miniaturized micro-positioner.

Fine wires have been tied around small bundles of dorsal or ventral root fibers and potted in Silastic silicone rubber. Such electrodes led to percutaneous connectors record action potentials from a number of single nerve fibers and permit fairly selective stimulation.

A multiple microelectrode holder and positioner has been developed and is reported under Project Report NDS(I)-69 LNLC/OC 1688.

The activities of Dr. Mishelevich during the present year have concentrated on the development of techniques for the separation of the single unit nerve impulses making up multi-unit records. This has proceeded through the development of computer programs for spike recognition, using either the NIH hybrid computer or the Micro-LINC computer in the NIMH/NINDS Section on Technical Development. The hybrid computer is a combination of a CDC-3100 digital computer paired with a Geospace SS-100 analog computer. The spike recognition system for this configuration was developed in collaboration with Mr. Stanley Selkow and Mr. John Rinzel of the Laboratory of Applied Studies of DCRT. It forms and separates clusters based on 1 to 7 amplitude points sampled per waveform exceeding a given threshold.

The Micro-LINC computer performs spike separation based on two variables selected from six possible combinations of amplitude and time. Spikes from as many as seven cells can be separated.

Major Findings: The hybrid computer spike recognition system has the advantage of allowing optimal sampling times to be determined iteratively by the machine and thus requires minimal operator intervention. It has the disadvantage, however, that it is off-line and cannot be directly connected to an on-going experiment. The Micro-LINC system is on-line and so will permit the results of spike recognition and separation to be used in experimental control.

Significance to Bio-Medical Research and the Program of the Institute: The development of chronic connections to the nervous system and analysis of neural activity present in such connections will produce two significant results. The first is that it will allow the study of basic motor control mechanisms in the intact animal. The second is that it will open the long-term possibility of making high resolution connections of prosthetic devices to patients.

Proposed Course of Project: The need for chronic connections to the nervous system is so basic to all phases of this Laboratory's objectives that this project is expected to continue from year to year with new approaches and results included as they occur. New methods for spike recognition will be explored and current methods evaluated further.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNLC/OC 1688
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cortical Mechanisms of Voluntary Motor Control

Previous Serial Number: None

Principal Investigator: Donald R. Humphrey, Ph.D.

Other Investigators: Karl Frank, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.0
Other:	0.3

Project Description:

Objectives: The objective of this project is to study the relationships between the activity of cortical neurons and the quantifiable aspects of simple motor acts. If the time-space patterns of cortical neuron firing are specifically correlated with graded motor behavior, such patterns could be used for the direct control of external devices. An additional objective is to determine to what extent the activity of cortical neurons is under voluntary control.

Methods to be Employed: Monkeys will be trained to perform simple motor acts such as flexion and rotation of the elbow and flexion-extension of the wrist against controlled opposing forces. Multiple microelectrodes will be adjusted to record trains of extracellular spikes from a number of neurons simultaneously, such as the pyramidal cells of the motor cortex. Cells will be selected for spike activity correlated with various components of the motor act. Patterns of spike activity will be analyzed by various techniques to determine whether or not the nature of the motor act can be predicted from the neural signals. In other experiments the animals will be rewarded for producing specific patterns of firing of cortical nerve cells in order to determine the degree of resolution of voluntary control which is possible.

Major Findings: This project is in the developmental stage and usable experimental data have yet to be obtained. A model of a new type electrode holder-manipulator has been developed which (1) is chronically implantable, (2) provides independent position control for a number of microelectrodes, and (3) is small enough so that the electrodes can all be positioned in a cortical volume of 1 or 2 $mm.^3$

Significance to Bio-Medical Research and the Program of the Institute: The information sought in this project could have important implications for current concepts of information processing by the central nervous system. In addition, the project represents a feasibility test to determine how patterns of multiple neuronal activity might be analyzed and transformed into useful signals for controlling an external device.

Proposed Course of Project: As described above

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LNLC/OC 1689
1. Laboratory of Neural Control
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electrophysiological Correlates of Learned Motor Acts

Previous Serial Number: None

Principal Investigator: Charles D. Woody, M.D.

Other Investigators: Karl Frank, Ph.D.

Cooperating Units: Section on Technical Development, NIMH/NINDS
Division of Computer Research and Technology

Man Years:

Total:	1.2
Professional:	0.9
Other:	0.3

Project Description:

Objectives: Electrical patterns of neural activity are sought which are correlated with the performance of learned motor acts and are sufficiently specific to provide a means for identifying at the cortex the initiation of particular movements. Such patterns will ultimately form a basis for operating prosthetic devices where normal mechanisms of effecting neural control of movement are impaired. They will also provide means for assessing deranged motor control as in apraxias and cerebral palsies. The initial objective is to determine whether electrical activity recorded from chronically implanted macroelectrodes contains such specific correlates for the simplest of movements. If so, knowledge of the nature of the specificity and of the physiologic mechanisms which control the initiation of movement can facilitate the definition of patterns of electrical activity associated with more complex movements.

Methods Employed: Cats are trained to perform simple movements such as an eye blink. Macroelectrodes are implanted chronically for periods of months in motor areas of the cortex. Recordings are analyzed with a Micro-LINC computer for patterns of activity correlated with the initiation of the learned movements. Analytic techniques include adaptive filter operations developed by Dr. Woody and others for identifying patterns of electrical

activity which have a variable latency of occurrence in relation to the initiating stimulus. These techniques serve as the basis for separating activity specifically related to movement from associated neural noise.

Major Findings: Recordings from multiple macroelectrodes in the orbital gyrus of the cat reveal specific patterns of activity associated with initiation of conditioned eye blinks. The activity precedes the contraction of the orbicularis oculi by the appropriate time required for conduction from cortex to periphery. Characteristic electrical responses are found for repeated eye closures and are discretely represented over cortical areas of several millimeters. The responses are largest in areas at which electrical stimulation through the recording electrode produces an eye blink. The lower the threshold required to elicit a blink, the larger the response. Comparable stimulus intensities at areas with smaller or absent responses elicit different movements. The findings indicate that specific patterns of electrical activity are associated with the initiation of simple movements and can be recognized by the methods employed.

Significance to Bio-Medical Research and the Program of the Institute: This investigation establishes for the first time patterns of gross electrical activity which can be related to the initiation of a specific movement and dissociated from non-specific factors, such as changing levels of fear or arousal. It provides a basis for further studies leading to the analysis of more complex patterns of movement.

Proposed Course of Project: Further studies of the specificity of these electrical responses will be performed by changing the afferent pathways mediating initiation of the conditioned motor act. Activity associated with different movements will also be investigated. Attempts will be made to distinguish individual responses from the on-going noisy background activity.

Honors and Awards: None

Publications:

Harris, E.K. and Woody, C.D.: Use of an adaptive filter to characterize signal-noise relationships. Comp. Biomed. Res. 2: 242-273, 1969.

HOW TO USE
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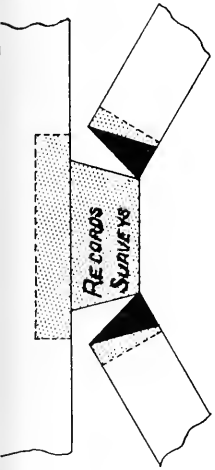

TABBED SEPARATOR SHEET
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(3-56)

Annual Report of the
Laboratory of Neurophysiology, Intramural Research
National Institute of Neurological Diseases and Stroke

July 1, 1968 through June 30, 1969

M.G.F. Fuortes, M.D., Chief

The Laboratory of Neurophysiology has not yet reached its final organizational structure due to problems dealing with space and personnel. In spite of these temporary difficulties, the operation of the Laboratory can be regarded as satisfactory.

Six scientists in the Laboratory have recently accepted positions in other units at the NIH: two in the Laboratory of Neural Control, NINDS, and four in the new NICHHD. Some difficulty is encountered in recruiting new scientists because the NICHHD has no Laboratory space, so that the four scientists due to move to that Institute will occupy space in our Laboratory for a period of perhaps 6-12 months after their transfer. Renovations of their Laboratory space will be completed as soon as the large backlog of work orders permits.

The Laboratory of Neurophysiology moved to Building 36 at the beginning of October 1968. Seven working units have been organized in the new quarters since that time and are now engaged in studies on receptors, central neurons and synapses. All projects are being investigated by electrophysiological techniques but there is close collaboration with other Laboratories where different methods are used, and new techniques are being developed for the study of cellular functions. For over a year the Ophthalmology Branch, NINDS, has performed morphological work in connection with our research on visual cells. Studies performed with optical and electron-microscopical techniques have contributed greatly to the interpretation of the physiological results. More recently, a collaboration has been started with the Laboratory of Biochemical Genetics, NHI. This Laboratory will study the chemical properties of certain preparations while the Laboratory of Neurophysiology will investigate their electrical and physiological features. These cooperative studies will involve the nervous system of simple invertebrates and the properties of nerves and muscles in tissue culture. Finally, the Laboratory of Neurophysiology receives considerable help from the Office of Mathematical Research, NIAMD and from the Laboratory of Technical Development, NIMH-NINDS, in problems involving computations, modeling and mathematical analysis of data. On this same subject, the continuing cooperation with the Department of Physics, University of Genova, Italy, deserves special mention. The Chairman of the Department served as a Special Consultant for our Laboratory in January 1969. During this time, a great

contribution to our research was made through advice on data analysis and on the interpretation of results. In addition, the Laboratory of Neurophysiology benefited by a very valuable series of seminars on the application of information theory to studies on the nervous system. These seminars have been attended by an enthusiastic audience of twenty NIH scientists. The lecture notes are now being prepared for distribution to interested workers at the NIH.

Two problems on the central nervous system of vertebrates have been investigated.

Three of the Laboratory's investigators have studied the synaptic organization of spinal motoneurons of cats. They found that slow motor units are powerfully controlled by the activity originating in muscle receptors, and are inhibited by cutaneous afferents. The fast motor units are less readily affected by afferent activity from muscle and are excited by cutaneous stimulation.

Two investigators are engaged in the analysis of the activity of auditory cells in the cortex of monkeys. The purpose of this study is to determine whether sounds which are meaningful for the animal in its normal life evoke some special type of response of cortical cells. Some units have been found which respond to the normal vocalizations of the monkey but cannot be activated by tones, clicks or white noises. These two projects will be discontinued at the end of FY 1969 because the scientists involved will move to different Laboratories.

Other studies on the organization of the nervous system are performed on invertebrates. An investigation was started in February 1969 to explore the structure and functions of nerve cells of round worms. This work is conducted in parallel with biochemical studies performed on the same animals by the Laboratory of Biochemical Genetics, NHI.

Another collaborative project involving biochemical and electrophysiological techniques is being conducted on tissue cultures. Dissociated mammalian nerve cells have been kept in culture for several weeks in the Laboratory of Biochemical Genetics, NHI. Most cultures seem to be contaminated by glia and attempts are now being made to obtain pure neuronal cultures. An investigator in the Laboratory of Neurophysiology has been successful in inserting microelectrodes into these cells and has measured their membrane potential, membrane resistance and spike potentials. The final aim of this study is to determine how electrical and chemical properties change as the cells develop, differentiate and form synaptic junctions.

The long-term effects of neural activity were studied at the neuromuscular junctions by three of our investigators. It was found that if a motor nerve is kept inactive for a few days or weeks, impulses release a larger than normal amount of transmitter. If a disused nerve is stimulated at 20-40 cycles per second, the transmitter output soon falls below control value, suggesting depletion of the available store of transmitter. Choline acetylase concentration is less in disused than in normal nerves.

The functions of receptor cells are investigated in different animals by other members of the Laboratory of Neurophysiology.

The work performed on visual cells of Limulus has the purpose of determining the relations between responses to light and active transport of ions across the photoreceptor membrane.

In the scallop, two types of receptor potential have been recorded from visual cells: a depolarizing response in cells of the distal layer and a hyperpolarizing response in the proximal layer.

A study of vertebrate photoreceptors has been completed, confirming previous reports that these cells are hyperpolarized by illumination. It was found that the hyperpolarization is accompanied by increased membrane resistance.

Work on visual cell responses has now reached a stage at which an attempt could be made to determine what molecular changes are associated to the visual process. The studies performed so far give reasons for believing that light changes the chemical activity of visual cells. It seems important to determine whether chemical changes correlated to visual responses may be detected by spectrophotometry. For this reason, an investigator is now developing a high-speed and high-resolution microspectrophotometer which will be applied to studies on visual cells. A crude prototype of this instrument has already been assembled and final construction will proceed as funds for the rather expensive components required will become available.

Serial No.:NDS(I)-58 LNP/OC-501
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Generation of Impulses in Nerve Cells

During FY 1969 this project was incorporated with Serial No. NDS(I)-69 LNLC/OC-1686.

Serial No.:NDS(I)-62 LNP/OC-934
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Basic Mechanisms of Synaptic Transmission

Previous Serial Number: Same

Principal Investigator: Robert E. Burke, M.D.

Other Investigators: Anders Lundberg, M.D.[1/]
Ladislav Vyklicky, M.D.
Pedro N. Rudomin, M.D.

Cooperating Units: 1/ Department of Physiology
University of Goteborg

Man Years:

Total:	2.5
Professional:	2.5
Other:	0.0

Project Description:

Objectives: Continuing work begun several years ago, this project has involved a study of the relations between properties intrinsic to motor units in large extensor muscles and the organization of synaptic input to the motor units. The basic objective in this has been to gain insight into the neural mechanisms which control the input-output transformations operating in postural versus voluntary movement. In addition to this main theme, several related problems involving the interaction of reflex systems in the spinal cord have been studied.

Methods Employed: All experiments were performed using adult cats. Conventional electrophysiological techniques were employed, including micropipette intracellular recording permitting current passage through the electrode. In addition, specialized instrumentation for recording muscle tension and for imposing force or displacement stimuli on the muscles under study has been developed.

Major Findings: Within the triceps surae motor unit pool, patterns of synaptic organization have been defined which are significantly related to the mechanical properties of the muscle unit portion of the motor units. In general, motoneurons innervating slowly contracting muscle fibers ("type S units") are more powerfully affected by afferent systems, both excitatory and inhibitory, which

are activated by muscle receptors, while motoneurons innervating fast muscle units ("type F units") are less tightly bound into such "automatic" systems. A different type of synaptic organization is apparent with respect to input from cutaneous afferents and from the rubrospinal tract; these input systems may effectively inhibit type S units and simultaneously excite the type F. Such synaptic patterns may underlie neural mechanisms permitting differential excitation of motor units specialized for "tonic" or "phasic" activity.

In a corollary study of spinal cord neural mechanisms, it was found that natural painful stimuli in the form of heat pulses caused powerful motor responses in the flexor reflex pattern despite concomitant primary afferent depolarization (or presynaptic inhibition) of cutaneous and group Ib afferent terminals.

Significance to bio-medical research and the program of the Institute: One of the basic questions in motor system physiology has concerned the differential control of reflex, or postural movements and of voluntary movements. The degree to which supraspinal neural systems utilize "pre-programmed" spinal reflex arcs in effecting complex behavioral movement is a question crucial to our understanding of volitional motor acts. The present project has been designed to investigate these questions by beginning with the final components of movement, the motor units, and investigating the synaptic organization which drives them.

Proposed Course of the Project: This project will be terminated at the end of FY 1969.

Honors and Awards: None

Publications:

Burke, R.E.: Group Ia synaptic input to fast and slow twitch motor units of cat triceps surae. J. Physiol. 196: 605-630, 1968.

Burke, R.E.: Firing patterns of gastrocnemius motor units in the decerebrate cat. J. Physiol. 196: 631-654, 1968.

Serial No.:NDS(I)-62 LNP/OC-973
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Integrative Mechanisms in the Central Auditory Pathway

Previous Serial Number: Same

Principal Investigators: Peter A. Winter, Ph.D.
H. Harris Funkenstein, M.D.

Other Investigators: Phillip G. Nelson, M.D.

Cooperating Units: Technical Development Section

Man Years:

Total:	2.0
Professional:	2.0
Other:	0.0

Project Description:

Objectives: In primates, vocal communication is very important for social interaction. This implies the analysis of complex auditory stimuli. It is likely that an essential part is accomplished at the level of auditory cortical neurons. As squirrel monkeys are highly vocal animals with well known vocal behavior patterns, this species was chosen for experimental analysis.

Methods Employed: In the first stage a platform molded out of metacrylate is attached to the skull. In a second stage the auditory cortical area is exposed with the dura matter left intact. A plastic cylinder is firmly anchored perpendicular to the cortical surface and fixed to the platform to guarantee additional support. After an initial sedative dose of 2mg nembutal, the monkey is restrained in a specially devised chair and the platform screwed tightly to a metal frame. The cylinder is filled with paraffin oil and an Evarts type extracellular recording system is mounted. For data analysis a MicroLINC computer is employed, generally programmed for a scanning procedure and for evaluation of spike frequency histograms. In addition to stimuli such as clicks, noise, pure and modulated (AM and FM) tones, complex tonal stimuli are presented such as pre-recorded squirrel monkey calls. The electrode positions are verified by histological examination.

Major Findings: So far only the superficial layers of the auditory cortex have been explored. The data obtained are not yet analyzed quantitatively. Nevertheless, the following general statements apply. Almost all neurons within the boundaries of the auditory cortex can be activated by some kind of acoustic stimulus. A variety of different response patterns were found. Inhibition (decrease of firing rate) was very often part of the response pattern. Rather broadly (several kc) as well as very sharply (less than 100c) tuned units were encountered. Some units were accurately timed to the on and off of a stimulus. Others could only be activated by noise or FM modulation. A minority of units responded only to complex acoustic stimulation such as pre-recorded calls.

Significance to bio-medical research and the program of the Institute: The knowledge of the functional organization of the auditory cortical area in primates is very fragmentary. Ablations result in deficit or total loss of an auditory discrimination task related to the amount of area removed and the difficulty of the task required. The findings are controversial. In humans impairment of a corresponding area within the left hemisphere causes severe and often dramatic deficits. We hope that microelectrode recordings with additional anatomical and behavioral studies may lead to a better understanding of the auditory cortical system in primates.

Proposed Course of Project: This project will be terminated at the end of FY 1969.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-65 LNP/OC-1239
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Photoreceptors in the Limulus Eyes

Previous Serial Number: Same

Principal Investigator: Thomas G. Smith, Jr. M.D.

Other Investigators: George C. Murray, Ph.D.
Joel E. Brown, M.D.[1/]
William K. Stell, M.D.[2/]

Cooperating Units: 1/ Department of Biology
Massachusetts Institute of Technology

2/ Laboratory of Neuropathology and
Neuroanatomical Sciences

Man Years:

Total:	4.0
Professional:	3.5
Other:	0.5

Project Description:

Objectives: The objective of this research is to gain insight into the means by which light is transduced into an electrical current in photoreceptors. The intracellularly recorded potentials of the photoreceptor cells of the horseshoe crab, Limulus polyphemus, are the object of study.

Methods Employed: The responses of the photoreceptor cells to light and electrical stimulation are recorded with intracellular microelectrodes and analyzed under a variety of experimental conditions. In this way, the role of visual pigments of the photoreceptor cell membrane and of the various ions in photoreception can be studied.

Major Findings: As mentioned in last year's Project Report, we have advanced the hypothesis that Limulus photoreceptor potential evoked by light is not the result of an increase in the cell's membrane permeability but is apparently due to changes in an electrogenic sodium pump. The major findings of the past year have been results which support the electrogenic sodium pump hypothesis.

These results were obtained in experiments where the effects of stimulation of the photoreceptor membrane with light or current were assayed in various intra and extracellular ionic environments and after treatment of the cells with various drugs known to affect the sodium pump.

Significance to bio-medical research and the program of the Institute: The experimental results and the attendant hypothesis have given further emphasis to the primary role of the photoreceptor membrane in the transducer mechanisms in visual processes. In addition, they narrow the hiatus in our knowledge of the steps involved between the absorption of light by rhodopsin and the electrical signals generated in photoreceptors. Thus, the main question of the transducer action in visual processes becomes the question of how the rhodopsin molecules, located in the photoreceptor membrane, affect those nearby membrane molecules that make up the sodium pump machinery.

Proposed Course of Project: It is proposed to continue the study of the generator potential and the electrogenic sodium pump in *Limulus* photoreceptors by undertaking experiments which will further test the hypothesis that alterations in sodium pump activity result in the generator potential. In addition, it is proposed to develop techniques which will allow simultaneous and independent measurement of the rhodopsin content of the cell by rapid microspectrophotometry and of the cell's visual responses by electrophysiology. Toward that end, considerable time and effort have been spent in surveying techniques which could be employed in a rapid scanning microspectrophotometer (See Project Report NDS(I)-69 LNP/OC-1690). With such techniques one may gain insight into how rhodopsin may regulate the sodium pump.

Honors and Awards: None

Publications:

Smith, T.G., Stell, W.K. and Brown, J.E.: Conductance Changes Associated with Receptor Potentials in Limulus Photoreceptors. *Science.* 162: 454-456, 1968.

Smith, T.G., Stell, W.K., Brown, J.E., Freeman, J.A. and Murray, G.C.: A Role for the Sodium Pump in Photoreception in Limulus. *Science*. 162: 456-458, 1968.

Nolte, J., Brown, J.E. and Smith, T.G.: A Hyperpolarizing Component of the Receptor Potential in the Median Ocellus of Limulus. *Science*. 162: 677-679, 1968.

Smith, T.G. and Baumann, F.: *Prog. Brain Res.*, Vol. 31, (In press).

Serial No.:NDS(I)-67 LNP/OC-1456
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Trophic Mechanisms in the Nervous System

Previous Serial Number: Same

Principal Investigators: Gerald D. Fischbach, M.D.
Norman Robbins, M.D.
Phillip G. Nelson, M.D.

Other Investigators: Mark Nameroff, M.D. 1/
Marshall Nirenberg, M.D. 2/

Cooperating Units: 1/ Air Force Institute of Pathology

2/ Laboratory of Biochemical Genetics
National Heart Institute

Project Description:

Objectives: The ultimate objective of this study is to determine the factors which underlie cellular plasticity or adaptability and synaptic specificity. Two main approaches were taken during the past year.

(1) The effects of chronic disuse on synaptic function were studied at the adult rat soleus neuromuscular junction. We chose the neuromuscular junction (the prototypic chemical synapse) to obtain direct measures of "synaptic efficacy" and chose the soleus muscle to take advantage of a reliable, relatively innocuous procedure for producing disuse. Our experiments were intended to answer some of the many questions raised by studies of disuse at various central relays.

(2) Membrane properties and synaptic relations were studied in muscle and nerve cells developing in tissue culture. An understanding of the detailed sequence of events in this system should help elucidate the controlling mechanisms in the intact, adult animal.

Methods Employed: (1) Disuse. Conventional intracellular microelectrode techniques; Linc computer for analyzing end-plate potentials.

(2) Tissue culture: Conventional intracellular techniques for recording and stimulating single cells. Muscle cells were derived from 11-d chick embryos and spinal cord explants from 5-11d chick embryos. Dissociated nerve cells were obtained from a variety of tissues including rat cerebellum. All cultures were maintained by standard techniques.

Major Findings: (1) Disuse - adult animal: Chronic disuse (3-days-6 weeks) had no effect on the spontaneous release of transmitter from nerve endings; the amplitude and frequency of miniature end-plate potentials (mepps) were unaltered. Transmitter released by nerve impulses was studied in the context of the quantum hypothesis of chemical transmission. Single impulses released a greater fraction (p) of the available store of transmitter at disused junctions. The mean quantum content, m = np, of a series of end-plate potentials (epps) was slightly increased and by inference, the available store of transmitter, n, was unchanged. The relative increase of transmitter released by disused terminals was maintained during periods of prolonged repetitive stimulation at frequencies at 5/s or 10/s. When "driven" at 20/s or 40/s, the transmitter output fell below control values.

Preliminary experiments in which the amount of nerve terminal choline acetylase was assayed with radioactive tracers, indicate that disused terminals contain less enzyme.

Facilitation was studied when transmission was blocked with Mg. The peak facilitation following single stimuli was unchanged, but following brief, conditioning tetani, a prolonged, "residual" potentiation was evident at disused junctions.

We could not produce excessive use at the soleus muscle (monitored with indwelling e.m.g. electrodes) failed. Attempts included (1) tenotomy of synergic muscles (2) forced standing on hindlimbs (3) forced walking exercises.

(2) Tissue culture. The resting membrane potential of initially dissociated muscle cells increases with total fiber length. The membrane resistivity is approximately the same as that of normal adult mammalian fibers and does not change during development. Action potentials were recorded from dissociated muscle and nerve cells. Evoked responses, presumably synaptically mediated, were recorded from explants of neural tissue which included spinal cord and dorsal ganglia.

Significance to bio-medical research and the program of the Institute: A study of the effect of disuse on neuromuscular transmission is an important step toward the understanding of the trophic relations between nerve and muscle. In addition, the results may have bearing on synaptic plasticity in the C.N.S. Modifiability of intercellular connections may in turn be the basis of learning

and memory considered in the broad sense. It may also lead to new approaches to the understanding and treatment of various chronic neurological diseases. The study of formation of synapses and development of differentiated, specific membrane phenomena in tissue culture may answer basic questions in developmental biology and also it may provide information about trophic phenomena in the nervous system.

Proposed Course of Study: In the past, we have studied the effect of chronic alterations in impulse activity on certain trophic phenomena between nerve and muscle (muscle contraction, synaptic transmission). In the future, we will further define the mechanisms of the changes observed at disused junctions (increased fractional release of transmitter and ultimately depression of transmitter output) and will assay the enzymes involved in the synthesis of transmitter. In addition, broader questions of neurotrophic influences will be approached with the techniques of tissue and organ culture.

This project will be terminated in the Laboratory of Neurophysiology, NINDS, at the end of FY 1969. These studies will continue in the Behavioral Biology Branch, NICHHD at the beginning of FY 1970.

Honors and Awards: None

Publications:

Fischbach, G. and Robbins, N.: Changes in Contractile Properties of Disused Muscle. J. Physiol. 201: 305-320, 1969.

Serial No.:NDS(I)-69 LNP/OC-1690
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Rapid High Resolution Microspectrophotometry in Visual Cells

Previous Serial Number: None

Principal Investigator: George C. Murray, Ph.D.

Other Investigators: Thomas G. Smith, M.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives: The objective of this research is to design, instrument and use rapid scanning microspectrophotometric techniques for the in situ investigation of the chemical kinetics of molecular processes within single excitable cells. Although these techniques will be developed for particular application to the molecular steps coupling photoexcitation of visual pigments to the excitation of the electrical response of the cells, their more general application to cellular physiology will be considered.

Methods Employed: To provide fast spectrophotometric monitoring of intracellular biochemical changes within single cells with a temporal resolution of better than 1 millisecond, a new microspectrophotometer has been designed which can sample transmittance at any spectral wave band between 350 and 600nm., either sequentially or in random order, at rates of 10 microsecond per sample point. The system is based on a rapid scan monochromator in which the entrance slit has been replaced by a cathode ray tube face. By moving the spot on the face appropriately the spectral output of the monochromator is unchanged. Furthermore, this allows the intensity to be modulated at high frequencies so that phase sensitive or coherent detection of the light transmitted can be used to improve the signal to noise ratio. An on-line digital computer will analyze the measured spectra and compute the concentration kinetics.

Major Findings: Based on a critical study of possible techniques of rapid spectrophotometry, the design mentioned above was selected for developmental evaluation. A prototype has been designed and partially instrumented from available components to test the basic principles at reduced speeds and within a restricted spectral range. Preliminary measurements indicate that the system is feasible and practical and that it should be further pursued.

Significance to bio-medical research and the program of the Institute: This project should provide the specific instrumentation and techniques for the measurement and analyses of the transduction steps which couple the stimulus to the electrical changes in the excitable membrane of photoreceptors. It will also provide a useful tool for similar study of other molecular system functioning within living cells, for which an increasing need has developed.

Proposed Course of Project: We propose to continue the development and construction of the prototype system. While the fast electronics and data analysis techniques are being developed, the optics and slower electronics will be used for initial investigations of the correlations between pigment changes and changes in the electrophysiologic properties of photoreceptor membranes, as discussed in report NDS(I)-65 LNP/OC-1239. Applications of these techniques to monitoring intracellular systems other than the visual pigments will be further explored.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/OC-1691
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Properties of Nerve Cells of Nematodes

Previous Serial Number: None

Principal Investigator: Eric A. Schwartz, M.D.

Other Investigators: Michael G.F. Fuortes, M.D.

Cooperating Units: Laboratory of Biochemical Genetics
National Heart Institute

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: The program is designed to elucidate the functional organization of a small nervous system.

Methods Employed: Nematode worms possess a denumerable and identifiable set of neural cells. All cells of the central nervous system can be identified and studied. Initial work is to determine microanatomy by intracellular fluorescent dye ionophoresis. With a complete nervous system map the physiology of discreet units will be accessible.

Major Findings: This research was started in February 1969. The principal advance has been the development of techniques for manipulating and studying these small animals. Several species have been investigated for suitability: Thorocostoma microlobatum, Mermis nigricans, Stephanurus dentalum, Ascaris sp. and Oxyuris equi. Oxyuris equi has been found to be most suitable.

Significance to bio-medical research and the program of the Institute: The work should contribute to our understanding of the principles of anatomic and functional organization of the nervous system. It should be possible to determine information transforms and to advance hypotheses of coding and decoding. The work supplements a study of biochemistry and genetics of the Nematode nervous system by the Laboratory of Biochemical Genetics, National Heart Institute.

Proposed Course of Project: With the techniques currently being developed it is intended to determine the microneural anatomy of Oxyuris equi. This will be determined by ionophoretic staining and electrophysiological techniques.

Honors and Awards: None

Publications: None

Serial No.:NDS(I)-69 LNP/OC-1692
1. Laboratory of Neurophysiology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of Responses of Retinal Cells in Vertebrates and Invertebrates
A. Vertebrate retina
B. Scallop retina

Previous Serial Number: None

Principal Investigators: Denis A. Baylor, M.D.
John S. McReynolds, M.D.

Other Investigators: Michael G.F. Fuortes, M.D.
Arnaldo Lasansky, M.D.[1/]
Anthony Gorman, Ph.D.[2/]

Cooperating Units: 1/ Ophthalmology Branch

2/ Laboratory of Neuropharmacology
St. Elizabeths Hospital

Man Years:

Total:	2.5
Professional:	2.5
Other:	0.0

Project Description:

Objectives: This research deals with the mechanisms by which electrical signals are generated and transmitted by cells in the retina upon stimulation by light. The main attention thus far has been devoted to the receptors, the first-order cells in the visual pathway, and the horizontal cells, which are synaptically activated by the receptors.

Methods Employed: The preparation employed for vertebrate studies is the retina of the turtle, chosen because the cells in it are relatively large, and the cellular architecture relatively simple. Microelectrodes have been used to record the activity of single cells during stimulation of the retina by light. Study of the fine structure of the turtle retina with the electron-microscope is being carried out by Dr. Lasansky. The scallop retina was chosen because it contains only photoreceptor cells whose axons form the optic nerve.

Major Findings: A. Vertebrate retina. The responses of individual photoreceptors to flashes of light have been studied. The site of recording has been verified by injecting a dye through the tip of recording electrodes and finding single stained photoreceptors (cones) in histological sections of the retina. The receptor response to a flash of light is a hyperpolarization whose size is graded with the intensity of the flash; the maximum amplitude is 15mV. In marked contrast to the behavior of the second-order cells, the receptor response to a tiny spot of light centered on the cell is the same as that to a large spot of the same intensity; this characteristic of the receptive field of the receptor response provides a convenient criterion for distinguishing the receptor response from that of other types of cells.

The graded hyperpolarizing response is the signal sent to higher order cells in the visual pathway; it is thus of interest to study the mechanism by which it is generated. In order to approach this problem, measurements have been made of the membrane resistance of the cell. It has been found that during the hyperpolarization produced by light, the resistance of the cell membrane increases markedly.

The increase in resistance of the cell membrane probably occurs in the outer segment, where light is absorbed; it appears to be due to a decrease in the ionic permeability of the cell membrane. Thus, the hyperpolarization produced by light is seen as being due to a reduction in the short-circuiting of the membrane in darkness. Further study has shown that when the membrane potential of the cell is set at a value near zero by the passage of a depolarizing current, the receptor response to light is abolished. If the inside is made positive with respect to the outside, the response is reversed in polarity. This result shows that the equilibrium potential for the ionic channel which light closes is near zero.

The observed relations between response size, light intensity, and membrane resistance change, can be explained by assuming that each absorbed photon generates a shower of particles which impinge on the outer segment membrane. The particles have the ability to inactivate ionic channels normally present in the membrane. The rate of inactivation is proportional to the number of channels present.

B. Scallop retina. The axons from the two types of visual cells in the scallop retina form the optic nerve, in which "on" and "off" discharges are recorded in response to light. Intracellular recording from the retinal cell bodies reveals two types of electrical responses to light. One type of cell gives a depolarizing response, and the other is hyperpolarizing. The two types of responses are segregated into two layers anatomically. With intracellular recording impulse activity is short-lived or

absent. However, when action potentials were observed in the hyperpolarizing type of cell, they were inhibited during illumination and returned with a transient overshoot in frequency when illumination ceased. The hyperpolarizing responses had a shorter latency of onset, which suggests that they are not post-synaptic to the depolarizing responses.

There appear, then, to be two separate types of photoreceptors in the scallop eye, and these receptors are capable of generating directly the "on" and "off" discharges in the optic nerve. This is in contrast to the vertebrate retina where such responses are the product of two or three stages of synaptic integration.

The results show that primary hyperpolarizing responses also occur in invertebrate visual cells. The hyperpolarizing cells in the scallop retina resemble vertebrate photoreceptors in having a ciliary structure, whereas the depolarizing scallop receptors resemble other invertebrate receptors, which have a microvillar structure.

The mechanism of the hyperpolarizing response may not be the same in vertebrate and invertebrate visual cells, however, as the hyperpolarizing response of the scallop cells seems to be associated with a decrease in membrane resistance, similar to that of inhibitory post-synaptic potentials.

Significance to Bio-medical Research and the Program of the Institute: One of the largest and most important gaps in our understanding of visual physiology concerns the basic mechanisms of receptor signaling. The results and their interpretation help to further our knowledge of how signals are generated and transmitted by the receptors.

Proposed Course of Project: We propose to study further the nature of the permeability change in the receptors and the mechanism by which absorption of light by a pigment is coupled to the permeability change. In addition, we would like to study the mechanism by which the receptor signal is transmitted to higher-order cells in the visual pathway.

In the scallop retina, it is planned to study further the electrical properties of both depolarizing and hyperpolarizing units to determine if the basic mechanisms are similar. We will attempt to mark histologically the individual units recorded from. We also plan to measure the spectral sensitivity of the two types of response as an independent method of confirming that there are two separate types of primary receptors.

Honors and Awards: None

Publications: None

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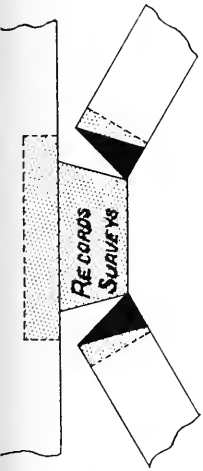

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

ANNUAL REPORT
July 1, 1968 through June 30, 1969
Laboratory of Biophysics, Intramural Research
National Institute of Neurological Diseases and Stroke

Robert E Taylor, Acting Chief

The book, Membranes, Ions and Impulses, published by Dr. K. S. Cole last year has received flattering reviews in Nature and Science and more than half of the first printing has been sold. A translation into Japanese has been completed by U. Kishimoto.

Considerable theoretical work has included studies of optimization of voluntary muscular control, effects of discrete versus uniform charge distribution on membrane surfaces, current and voltage fluctuations (noise) in natural and artificial membranes and repetitive firing and adaptation in nerve models. A number of theoretical predictions of the Hodgkin-Huxley equations have been compared with experimental work done in the laboratory: for squid axons treated with NH_4 or D_2O; membrane current in response to linearily changing membrane potentials (ramp clamp); action potential interruption with a new system for electronic switching from current to voltage clamp. These studies have been facilitated by ready access to a remote computer station in Bldg. 36 and the development of methods for producing graphs from data on punched paper tape.

Many of these studies have and will continue to be done with squid axons in Woods Hole and Chile but the use of the central nerve cord of the marine worm Myxicola infundibulum, a preparation developed in this laboratory, has now become routine for voltage clamp studies and techniques for axoplasm replacement and perfusion are being devised. Data has been taken using the LINC computer and programs are being developed for processing both off and on line. A program has been started for measurements of noise fluctuations in the membrane of the crayfish claw muscle. This preparation does not have the annoying external layer of Schwann cells and connective tissue making isolation of a small patch of membrane difficult using squid or lobster axons. As yet no satisfactory common basis has been found for systems which show predominately slow fluctuations including semiconductor and nerve membrane electrical noise.

A statistical theory has been derived to account for the fluctuations of conductance in EIM (Excitatory Inducing Molecule) treated lipid bilayers with opening and closing of microscopic unit conducting channels.

The only justification for the use of the classical formulas of Maxwell and Rayleigh for conductance of suspensions of spheres and cylinders at high volume concentrations relevant to interpretations of measurements on tissues has been supplied by approximate analyses and measurements of analogues (conducting paper for triangular, square and hexagonal arrays of cylinders and electrolytic tank measurements on cubes and 14-hedra).

After twelve years of repeatedly returning to the question it appears that the effect of calcium ions on the squid axon membrane may be limited to a cation selective leakage conductance along with the known shift of the apparent membrane potential. There is no clear evidence that calcium interacts with the normally potassium conducting channels. A model for the explanation of the membrane potential shift yields values for the density of fixed charges in the vicinity of the potassium channels and the equilibrium constant for binding of divalent ions to these sites.

Proton magnetic resonance studies have been extended to include myelin, erythrocyte and E. coli ghosts, and fragments of squid retinal nerve membranes obtained from Chile. The hydrocarbon part of these preparations have all been found to have about the same fluidity. The thermally induced structural changes detected in E. coli ghosts is correlated with a change in the membrane permeability to sugar phosphates. Paramagnetic products of photolysis of enzyme bound cobalamin (being studied by Babior, NHI) were identified by electron spin resonance (EPR). Our EPR spectrometer is now in full operation and studies are being made using a rhodopsin lipid complex which has been isolated by Shichi and Lewis (NINDS, Laboratory of Ophthalmology). Work on the mechanism of charge transfer in certain organic semiconductors is planned.

Conductance fluctuations in synthetic bilayer membranes treated with EIM are being studied and individual unit conducting channels have been observed to be either open or partially closed, and account for the observed negative (slope) conductance.

Serial No. NDS(I)-62 LB/CB 935
1. Biophysics
2. Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ionic Permeabilities of Excitable Membranes. Electrical Experiments and Analyses.

Previous Serial Number: Same

Principal Investigators: Cole, K.S., Ph.D. and Binstock, L., B.S., M.S.

Other Investigators: Fishman, H., Ph.D., Goldman, L., Ph.D., Li, C.-L., M.D., Ph.D., and Bak, A. F.

Cooperating Units: University of California, Berkeley, Calif.
University of Maryland, College Park, Md.
Surgical Neurology Branch, NINDS, NIH
Laboratory of Neurophysiology, NIMH

Man Years

Total:	4.0
Professional:	3.0
Other:	1.0

Project Description:

Objectives: The ionic current flow across the membrane of the squid giant axon and the nerve cord of Myxicola have been measured, without the complications of excitation and propagation, after a sudden change of the membrane potential. The currents have been analyzed in terms of the membrane permeability to sodium and potassium ions. Much of classical nerve physiology is explained by these permeabilities which are themselves not understood. The long range objectives are the further interpretation of nerve function in terms of these fast ionic permeabilities and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Measurements of the ionic current flow across the membrane of the giant axon of the squid and the nerve cord of Myxicola, without the complications of excitation and propagation have been improved by the simultaneous use of replacement of internal contents and continuous perfusion of known solutions.

A ramp voltage clamp system has been used to produce on-line I-V curves from Loligo and Myxicola axons. These data are then compared to the computed curves for ramps using the Hodgkin-Huxley formulation.

Major Findings: The new Book, "Membranes, Ions and Impulses" by Dr. Kenneth S. Cole received highly flattering reviews in Nature and Science. The University of California Press reports that more than half of the first printing have been sold. U. Kishimoto has completed a translation for a Japanese edition in two volumes which should appear within a year.

Myxicola infundibulum, a marine burrowing sand worm is available in the Laboratory on a regular basis all year-round. The dissection procedure for preparing the Myxicola giant axon (which is most of the nerve chord) for voltage clamp now takes only about 45 minutes. Mean values for axon diameter, resting membrane potential, action potential, maximum peak inward transient current, and resting membrane resistance are 550 microns, -67 mv, 112 mv, 0.90 mA/cm^2, and 1.2 $K\Omega cm^2$ respectively. This preparation, under voltage clamp, is extremely stable in time lasting as long as five hours. Cut branches do not seem to be a problem. Tetrodotoxin (TTX) blocks the action potential and both inward and outward transient currents, but has no effect on the resting potential or the steady state current. The selectivity of TTX on the transient current indicates that this current is carried by sodium.

The effect of reducing the external sodium concentration on resting potential, action potential, membrane current, and transient current was studied in the Myxicola giant axon. Tris chloride was used as a substitute for sodium chloride. The transient current is carried mostly by sodium while the delayed current seems to be independent of external sodium. The transient current reversal potential behaves much like a pure Nernst equilibrium potential for sodium. The action potential varied with external sodium as expected. Resting membrane potential did not change with external sodium.

Early leakage currents, for times similar to the time to peak of the transient current, were measured in the Myxicola giant axon in the presence of tetrodotoxin. The leakage current-voltage relation rectifies, showing more current for strong depolarizing pulses than would be

expected from symmetry around the holding potential. A satisfactory practical approximation for most leakage corrections is constant resting conductance. The leakage current-voltage relation can be fitted by summing the constant field current for potassium with a constant conductance component. It appears to be that the leakage current is determined by at least three ionic components.

The axoplasm of the Myxicola giant axon has been removed by means of a long infusion cannula. The internal perfusate used was 0.5 M KF, but no action potential was obtained. The axon seems to handle very well and appears to withstand the stress of the long cannula and electrodes.

A slow rising ramp (0.5 V/sec) obtains an I-V relation which is a good approximation of a late steady-state (potassium) relation. Similarly, a fast rising ramp (50 V/sec) preceeded by a 30 mV hyperpolarizing step prepulse gives an approximate fast transient (sodium) I-V relation. The kinetics of the slow potassium inactivation in high external potassium solutions has been recorded using the slow ramp to follow the alterations in the potassium I-V characteristic.

Many measurements have been made for many years on the passive electrical characteristics of many tissues under many conditions. Empirical correlations of the electrical conductance with other observations have shown a probable importance in many problems--such as muscle excitation, circulation, anesthesia, spreading depression, edema and even learning and memory. Such measurements have often been interpreted by the classical formulae of Maxwell and Rayleigh for the volume concentration of non conducting spheres and cylinders. But for resulting values of 70% to 95% the use of these equations had no justification. Continuing work started about 1930, approximate analyses showed that the equations were indeed valid as various space-filling forms were slightly separated. Subsequent measurements with conducting paper analogues for triangular, square and hexagonal cylinders showed that the Rayleigh equation applied over the entire range from zero to 100% volume concentration. Similarly electrolytic tank measurements on cubes and 14-hedra followed the Maxwell equation over the same range. Thus these analogue solutions of the fundamental potential theory problems give a justification--and the only justification--for the application of the classical equations to the interpretation of measurements on tissues.

Another analysis showed that in the usual range of membrane characteristics the cells may be considered to be nonconductors as was used in the analogues.

Scientific Significance: A thorough understanding of the relationships between ionic concentrations, inside and outside of the cell and the dependence of the ionic currents on potential are necessary to understand the normal functioning of the excitable system as part of the attempts to elucidate the molecular structure of the membrane.

The Hodgkin-Huxley (HH) formulation presently gives the most complete description of axon behavior. Unfortunately, the HH parameters are obtained only after tedious processing of step voltage clamp data, and therefore are usually not determined. Instead, there has been an increasing tendency to draw conclusions from the current-potential relations for the late steady state and early transient maxima which under normal conditions give the potassium and sodium conductance characteristics. There have been important enough conclusions drawn from such a presentation of step clamp data to thoroughly justify this procedure. The ramp voltage clamp appears to be a means for obtaining these data as directly and expeditiously as possible.

Proposed Course of Project: Collaborative projects with the University of California at Berkeley and the University of Maryland will continue.

The Myxicola giant axon is similar in all its essential features to the squid giant axon and it survives space-voltage clamping very well. The next step of achieving a satisfactory simultaneous internal perfusion and voltage clamping of the axon from Myxicola is being vigorously attacked and is expected to be solved. This will make it possible to do experiments in Bethesda which are now possible only during field trips.

The rapid and direct presentation of I-V relations is particularly appropriate in studying the kinetics of various substances which alter membrane permeability. An attempt will also be made to simultaneously measure infinite frequency (chord) conductance and I-V characteristics, to give a fairly complete description of membrane behavior.

Some aspects of the limitations of membrane conductance and measuring frequency need to be clarified and a more complete explanation of the observed tissue impedance behavior is a remaining problem. Such further analogue calculations will be undertaken when possible.

Honors and Awards: None

Publications:

Cole, K. S., Li, C.-l., and Bak, A. F.: Electrical analogues for tissues. Experimental Neurology (in press).

Serial No. NDS(I)-62 LB/CB 939
1. Biophysics
2. Cellular Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ionic Permeabilities of the Squid Giant Axon Membrane. Electrical Experiments and Analyses with Alterations of Environments.

Previous Serial Number: Same

Principal Investigators: Gilbert, D.L., Ph.D. and Taylor, R. E, Ph.D.

Other Investigators: Ehrenstein, G. M., Ph. D., Stillman, I., M.D., Ph.D., Rojas, E., Ph.D., Bezanilla, F., Ph.D., and Atwater, I.

Cooperating Units: Marine Biological Laboratory, Woods Hole, Massachusetts
Laboratorio de Biologia Celular, Montemar, Chile

Man Years.

Total:	3.2
Professional:	3.0
Other:	0.2

Project Description:

Objectives: To study the ionic current flow across the nerve membrane without the complications of excitation and propagation in terms of individual ion currents and to observe the effects of changes in normal internal and external ionic concentrations. The long range objectives are the interpretation of nerve function in terms of ion movements and the elucidation of the structures and mechanisms by which the permeabilities are controlled.

Methods Employed: Standard voltage clamp techniques are employed with the addition of a control of the internal environment by replacement and perfusion. Some of the data are fit to a mathematical model with computer techniques.

Major Findings: Using the data for potassium currents in squid giant axons immersed in isosmotic potassium chloride with varying concentrations of calcium and magnesium it was calculated, in terms of a model formulated here that the average distance between external negative fixed charges in the vicinity of the potassium "channels" was about 10 angstrom units. This model also yielded a value of 0.1/M for the equilibrium constant for the binding of divalent ions to these fixed charges.

Observations were continued on the depolarizing effects of redox substances (potassium ferri- and ferrocyanide). Both sodium and potassium currents are decreased. The effects are reversible for short periods of exposure.

Monactin, a cyclic polypeptide reported to induce a potassium specific permeability increase in mitochondrial, erythrocyte and artificial membranes, was tested on the squid axon. The finding was a depression of the normally occurring potassium current which appeared to be due to the n-octanol in which the monactin was dissolved. There is a strong suspicion that the n-octanol is also involved in the reported effects on artificial membranes. In one experiment valinomycin had no effect on currents in the Chilean squid axon.

With further experiments and analyses on the effects of external calcium and magnesium on the squid axon membrane perfused with KF and bathed in KCl it appears that while the shift on the voltage axis of all measured parameters is real there are no other effects which can be ascribed to any action of divalent ions on the potassium channels. There is a reversible increase in a cation selective leakage in the absence of divalent ions and later (about one-half hour) an irreversible increase in a non-specific leakage component of membrane current.

On suddenly increasing the potassium concentration in the squid axon, either externally or internally, the resting membrane potential changes to a new value with two exponential time constants of about 10 and 60 seconds. Calculations for the effects of diffusion yield results which are consistent with the 10 second time constant. The 60 second time constant remains unexplained, but is similar to the time course of the effects of sudden changes in external calcium.

A new method was developed for determination of the

membrane conductance during an action potential. This was done by initiating an action potential under conditions of zero membrane current and suddenly switching to a voltage clamped condition at some predetermined potential. Measurements of the current steps for a number of different potentials gives the "instantaneous" current voltage curve at some time during the action potential. The curve turned out to be linear and gives the instantaneous conductance and Thevenin potential and, with less accuracy, the membrane capacitance. The action potential interruption procedure was initially desired for the purpose of measuring the time course of sodium and potassium currents during the action potential with radioactive tracers (by Bezanilla, Rojas and Atwater).

Scientific Significance: Electrokinetic determinations which have appeared in the literature indicate the presence of a negative fixed charge for the squid axon but these charges must clearly be residing on the surface of the connective tissue layer present. The results given in this report do not show conclusively that the observed phenomena are due to fixed charges but if they are, which is likely, they give the density of these charges and the binding capacity for divalent ions.

The influence of redox substance on the membrane should provide valuable clues to the location of effected chemical groups. The so far negative results with cyclic polypeptides are consistent with the supposition that clinically used antibiotics are not likely to have a profound action on nerve cell membranes.

Proposed Course of Project: Further experiments and analyses are in progress or planned to investigate the nature of the surface charges and to increase our knowledge of the phenomonological effects of changes of internal and external ion concentrations on nerve membranes in an effort to expand the basis for a rational attack on the molecular mechanisms involved.

Honors and Awards: None

Publications:

Gilbert, D. L., and Ehrenstein, G.: Effect of divalent cations on potassium conductance of squid axon: determination of surface charge. Biophys. J. 9: 447-463, 1969.

Gilbert, D. L.: The interdependence between the biosphere and the atmosphere. Respir. Physiol. 5: 68-77, 1968.

Gilbert, D. L.: Geo-biological exchange: dynamics of CO_2 exchange between biosphere, hydrosphere, lithosphere and atmosphere. Proc. Internat. Union of Physiol. Sci. 6: 179-180, 1968.

Serial No. NDS(I)-62 LB/I 940
1. Biophysics
2. Instrumentation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ionic Permeabilities of Excitable Membranes: Effects of Chemical Agents.

Previous Serial Number: Same

Principal Investigators: Binstock, L., B.S., M.S., Lecar, H., Ph.D.
Stillman, I., M.D., Ph.D.

Other Investigators: None

Cooperating Unit: Marine Biological Laboratory,
Woods Hole, Massachusetts

Man Years

Total:	1.4
Professional:	1.1
Other:	0.3

Project Description:

Objectives: The voltage clamp technique allows measurements of ionic current flows across nerve membrane as a function of voltage and time. These parameters have been combined into a set of empirical equations capable of expressing nerve membrane activity, but the physical meaning of the equations is unknown. It is hoped that the use of various chemicals known to effect nervous activity may contribute to understanding the physical mechanisms of the membrane. Irrespective of the chemical properties of the substances, this may permit dissection of the lumped parameters of the equations as well as a correlation of known chemical properties with the observed effects.

Methods Employed: The voltage clamp technique used in these experiments were described previously. For the internal perfusion experiments, modifications to the Tasaki suction perfusion technique were made to allow the use of the voltage clamp technique. This required inserting the coaxial electrode, which consists of an axial current electrode and a potential measuring probe, into the

suction or outflow cannula without clogging the perfusion flow, a task much more difficult for the Woods Hole axon than the Chile axon, because of the smaller size of the Woods Hole axons.

To make this task less difficult, another modification was made which consisted of using two separate electrodes instead of the single coaxial electrode. The axial current carrying electrode was attached to the infusion cannula and a smaller potential measuring electrode was inserted into the outflow cannula.

Major Findings: Further analysis has confirmed that the ammonium ion carries the early transient current with 0.3 times the permeability of sodium and it carries the delayed current with 0.3 times the potassium permeability. The conductance changes observed in voltage clamp shows approximately the same time course in ammonium solutions as in the normal physiological solutions. These ammonium ion permeabilities account for the known effects of ammonium on nerve excitability. An NH_4 action has been computed from the Hodgkin-Huxley (1952) equations using the NH_4 permeability values determined from the voltage clamp data. The theoretical action potential agrees well with the observed NH_4-action potential without the necessity of modifying any of the time constants or normalized conductance functions in the Hodgkin-Huxley equations. The only parameters changed were the absolute values of the conductances and the reversal potentials. The effects of D_2O have been further studied and the investigations have been extended to include the use of the giant axon preparation from Myxicola.

Proposed Course of Action: It is planned to study more "sodium-substitutes" and "potassium-substitutes" of the quaternary-ammonium ion type and others if they can be found to see what their effect is on the nerve membrane. To increase the understanding of the effects of heavy water, experiments are planned with variations in D_2O concentrations, ionic strength, temperature and pH. These studies will be continued with the squid giant axon and extended to the axon of Myxicola.

Honors and Awards: None

Publications:

Binstock, L. and Lecar, H.: Ammonium ion currents in the squid giant axon. J. Gen. Physiol. 53: 342-361, 1969.

Serial No. NDS(I)-62 LB/MA 936
1. Biophysics
2. Mathematical Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ionic Permeabilities of Nerve Membranes: Theoretical Investigations.

Previous Serial Number: Same

Principal Investigator: FitzHugh, R., Ph.D.

Other Investigators: Cole, K. S., Ph.D., Lecar, H., Ph.D., Nossal, R., Ph.D., Shaw, J.,B.S.,M.A.,and Bezanilla, F., Ph.D.

Cooperating Unit: Division of Computer Research and Technology, NIH
Section on Technical Development, NIMH,NIH
Laboratorio de Biologia Celular, Montemar, Chile

Man Years

Total:	3.2
Professional:	3.0
Other:	0.2

Project Description:

Objectives: To formulate and test theoretical descriptions of (1) excitable ion-permeable membranes, including statistical fluctuations, and (2) voluntary muscular control, using mathematical analysis and computation.

Methods Employed: Mathematical analyses, with particular emphasis on nonlinear differential equations and optimization theory, are being used in conjunction with the available computing facilities.

Major Findings: Computations have shown that the Frankenhaeuser-Huxley differential equations for the amphibian node show impulse trains over a much narrower range of values of constant current stimulus than the Hodgkin-Huxley equations for the squid giant axon. An analysis of the stability properties of the singular point

corresponding to the stationary state during constant current flow has been started, with the hope of identifying those elements of the HH and FH equations which are primarily responsible for the presence or absence of impulse trains. This analysis should be useful in developing a model of adaptation, a phenomenon which is not represented in either the HH or FH model.

The theory of fluctuation phenomena in excitable membranes and their effect on threshold fluctuations, based on the Hodgkin-Huxley equations, has been further developed (in collaboration with Dr. R. Nossal of DCRT). The theory predicts correctly the shape and width of the curve of probability of firing versus stimulus intensity, as well as the fact that this width is directly proportional to the threshold value of stimulus. It also clarifies the apparently paradoxical fact that a membrane with fluctuating conductance can exhibit relative fluctuations in threshold smaller in magnitude than those in conductance.

The conductance fluctuations in EIM-treated synthetic membranes, observed experimentally in this laboratory appear to be caused by the opening and closing of many microscopic unit conducting channels. A statistical theory has been derived to explain these fluctuations, in which the observed macroscopic quantities, such as the current-voltage relation and the noise-power spectrum, are related to microscopic quantities such as the activation energy of opening of a single channel and the degree of cooperative interaction between channels.

A theoretical action potential was computed from a modified HH model, in which the ionic conductances and reversal potentials were changed in accordance with voltage clamp data from a squid axon treated with NH_4^+. The computed curve agrees well with the experimentally observed action potential and was published as Fig. 9, in L. Binstock and H. Lecar, J. Gen. Physiol. 53:342, 1969.

Many membrane phenomena may depend upon surface charges which are usually expressed as a uniform density. An elementary analysis has been made of the potential near or centered between ionic charges in a triangular surface array, in terms of ion spacing and the ionic strength of the bounding electrolyte. The ion spacing calculated for a given potential near an ion is less than for the same potential midway between nearest ions. This in turn is less than for a spacing equivalent to the

potential for a uniform charge distribution. The interpretations of such calculations in terms of membrane structure and function are not yet sufficiently certain to give the differences between these three distributions a useful significance.

Computations of membrane current of a squid axon in response to linearly changing membrane potentials (ramps) have been made, using the HH equations. These simulations made it possible to search for proper ramp slopes for directly obtaining experimental on-line current-potential characteristics, without need for data analysis, of either the fast (sodium) or steady-state (potassium) process. A manuscript entitled "On-line measurement of axon membrane current-potential characteristics" by H. Fishman and K. S. Cole, which describes the experimental curves thus obtained, is in preparation.

An analysis was made of current transients as predicted by the HH equations for the usual voltage clamp steps and for sudden interruption of zero current action potentials to a voltage clamped condition by modifying the computer program developed by FitzHugh to include the effects of a resistance in series with the nerve membrane and a non-instantaneous voltage clamp feed back loop.

The modeling of voluntary muscular activity, using a hypothesis of optimal control, has been extended. The general results obtained last year have been substantiated in greater detail, for more combinations of the four basic types of muscle load: constant, elastic, viscous, and inertial forces and for pairs of antagonists as well as for single muscles. Optimal control theory, nonlinear mechanics, and computations have been used to provide proofs of results and to illustrate detailed differences arising in different cases. Work has been started on a manuscript describing these results, including an extensive examination of the physiological literature on voluntary motion and its optimization.

Methods for producing graphs from data punched on paper tape by the teletype remote computer station have been developed, with the cooperation of the Section on Technical Development, NIMH. The old LINC in Building 10 has been programmed to read in the paper tape and produce graphs on the Benson-Lehner digital plotter. An interface between a paper tape reader and the Micro-LINC which will permit more convenient plotting on the Calcomp plotter in Building 36, is nearing completion.

Scientific Significance: Noise and fluctuation phenomena in nerves serve both as a means of learning about the membrane conduction processes underlying excitation and also for assessing the limitations of axons as transmitters of information. The theoretical study of the conduction process in the treated synthetic membrane serves to clarify a number of the notions about conducting channels in excitable membranes which have been proposed to explain the voltage-dependent permeability.

Membrane stability and the conditions under which impulse trains occur are relevant to an understanding of slow excitability changes and adaptation, which are of general importance in the nervous system, particularly in sensory neurons.

The hypothesis of optimality in modeling voluntary muscular activity provides a potentially useful theoretical approach which does not require a detailed knowledge (still lacking) of the functioning of the CNS in muscular coordination. The hypothesis is made plausible by the concept of evolutionary selection of CNS functioning which tends to favor survival.

Proposed Course of Project: The study of both membrane stability and membrane fluctuations will be pursued, with the aim of improving present mathematical models of excitable membranes, which are inadequate in these respects.

The theory of fluctuation phenomena in excitable membranes is being extended to take into consideration different possible physical noise sources and to study spike interval fluctuations.

It seems highly probable that interpretations of some electrokinetic data on single axons have been incorrect and should not be used as a basis for a membrane model. Evidence for this negative conclusion may be difficult to obtain but limited cooperation with Berkeley and Cambridge may be useful.

The solution of the equations for optimal muscular control will be sought for a variety of mechanical constraints acting on a single muscle, a pair of antagonists, or more complicated musculoskeletal systems. It is hoped that the results may provide at least qualitative predictions for comparison with experiment and the eventual production of a coherent theory of coordinated motion.

Honors and Awards: None

Publications:

Cole, K. S.: Zeta potentials and discrete vs uniform charge distribution. Biophys. J. 9: 465-469, 1969.

FitzHugh, R.: Motion picture of nerve impulse propagation using computer animation. J. Appl. Physiol. 25: 628-630, 1968.

Serial No. NDS(I)-62 LB/ME 938
1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Membrane Dielectric Properties.

Previous Serial Number: Same

Principal Investigator: Cole, K.S., Ph.D. and Taylor R. E, Ph.D.

Other Investigators: None

Cooperating Unit: None

Man Years

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: The need for more and detailed measurements of membrane dielectric properties remains, particularly in the low frequency range and the effects of temperature and possible drug and ion effects.

Methods Employed: The presently available methods for space and voltage clamping the squid giant axon are required as well as the developing techniques for internal replacement and perfusion and the use of accurate bridge networks.

Major Findings: The history and present status of membrane capacity measurements and mostly inadequate analyses and theories were summarized for a conference on physical principles of biological membranes.

Scientific Significance: Detailed information on the impedance of the membrane, particularly the voltage and temperature dependence relations, imposes constraints on any molecular model which is proposed. One example is that the membrane does not have the same dielectric properties as the simple bilipid layers which have been worked on.

Proposed Course of Project: Further experiments are planned

and will be done when the possibility presents itself. The techniques are available but the squid are to be had only at Marine Biological Stations. Transportation of squid appears to be feasible but the development of the necessary facilities are beyond the budgetary and manpower resources of the Laboratory.

Honors and Awards: None

Publications:

Cole, K. S. Dielectric properties of living membranes. In Perlmutter, A. (Ed.): Proceedings of the Coral Gables Conference on Physical Principles of Biological Membranes (in press).

Serial No. NDS(I)-65 LB/ME 1240
1. Biophysics
2. Membrane Biophysics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Molecular Biophysics - Physical Properties of Membranes and Simple Membrane-like Systems.

Previous Serial Number: Same

Principal Investigators: Ehrenstein, G., Ph.D., Lecar, H.,Ph.D., Stillman, I. ,M.D., Ph.D.

Other Investigators: Nossal, R., Ph.D., Mackey, M., Ph.D., and Canessa, M., Ph.D.

Cooperating Units: National Bureau of Standards
Division of Computer Research and Technology, NIH
Laboratorio de Biologia Celular, Montemar, Chile

Man Years

Total:	2.2
Professional:	2.0
Other:	0.2

Project Description:

Objectives: To study physical properties and molecular structure of well-defined synthetic systems related to natural membranes. To examine related molecular properties of excitable membranes with newer physical techniques.

Methods Employed: Synthetic bilayer membranes are prepared from beef brain extracts, oxidized cholesterol and other liquids between two KCl solutions. The electrical properties of the membranes are measured before and after the application of an activating factor (EIM, probably protein and other substances) by means of calomel electrodes and low noise-high resistance amplifiers.

Proton magnetic resonance spectroscopy is used to determine the internal structure of the hydrocarbon moiety

of isolated biological membranes, such as nerve myelin sheath, squid retinal nerve, erythrocyte ghosts and E. coli ghosts.

Electron spin resonance spectroscopy is used to study photoexcited states and photochemical products of biological interest.

Major Findings: On addition to, or incorporation in, synthetic bilayer membranes of a bacterial produced "activating factor" the membrane conductance increases in small steps of about 0.5 nanomhos. Application of the proper potential across the membrane causes the conductance to decrease, again in small steps. For a fixed potential similar random conductance fluctuations occur. It has been found that with as few as twenty channels the current voltage curve for the membrane is non-linear with a pronounced negative resistance region while the conductance of the individual channels is ohmic.

The nuclear magnetic resonance studies of myelin have revealed that the hydrocarbon moiety has a high mobility and behaves like a rather viscous liquid, in agreement with recent findings on the mobility of metabolites in myelin. Preliminary experiments using E. coli membranes (in collaboration with Dr. R. Kaback of NHI, NIH) show a correlation between thermally induced changes detected by NMR and thermally induced changes in permeability to sugar phosphate.

Preliminary wide line NMR experiments with isolated fragments of Chilean squid retinal nerve membrane showed no difference in fluidity as compared with extracted myelin.

As an adjunct to the work of Dr. B. Babior (NHI, NIH) on the mechanism of the catalytic action of vitamin B_{12} the paramagnetic products of photolysis of enzyme-bound alkyl cobalamins were determined by electron spin resonance.

Scientific Significance: A major and fundamental question concerning ionic permeability of natural membranes is whether there are discrete channels which individually have a continuous voltage dependent conductance or whether specific conducting sites are either open or closed. For the case studied here with synthetic lipid bilayers it is increasingly clear (by direct measurement) that the total conductance is the statistical result of individual channels which are ohmic and either open or (partially)

closed. Thus for this system a large class of suggested membrane models may be discarded as irrelevent because the fundamental unit for conductance is not voltage dependent, only the proportion of time during which it is open.

Interest in membranes is not limited to electrical conductance and transfer of materials as they appear to be involved in enzyme activity, macromolecular synthesis, release and effects of hormones, antibody production and immulogical responses. The importance of increasing our knowledge of these membranes and simpler membrane like systems is great indeed. Nuclear magnetic resonance studies of isolated membrane preparations provide information about the structure and fluidity of the interior of the membrane. In particular the studies of myelin produce a means for discriminating between the various models which have been proposed to explain the remarkable stability of the myelin sheath and the breakdown of this stability in certain diseases such as multiple sclerosis.

Proposed Course of Project: Properties of individual conducting channels in synthetic bilayers will be further investigated and channel density under various conditions determined by 1) division of membrane conductance by channel conductance and 2) by analysis of the fluctuation (noise) of conductance. Comparison of the two results will be one way to investigate independence and homogeneity of separate channels.

Artificial membranes will be employed in a number of studies and as time and circumstances allow such as attempts to incorporate photosensitive pigments beginning with the rhodopsin-lipid particle preparation which has been extracted from retinal rods by Shichi and Lewis (NINDS,NIH).

Further studies will be made in correlating information from nuclear and electron spin resonance spectroscopy. The development of membrane permeable organic free radicals allows the possibility of studying the fluidity of the membrane interior with the EPR machine (so-called spin labelling).

Honors and Awards: None

Publications:

Babior, B., Lecar, H. and Kon, H.: The photolysis of enzyme-bound alkyle cobalamins. Biochemistry (in press).

HOW TO USE
THESE SEPARATORS

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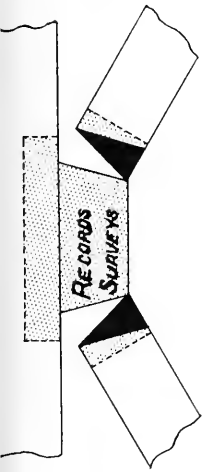

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

Annual Report of the
Laboratory of Experimental Neurology, Intramural Research
National Institute of Neurological Diseases and Stroke

July 1, 1968 through June 30, 1969

William F. Caveness, M.D., Chief

The Laboratory has been maintained at Columbia University, College of Physicians and Surgeons by contract mechanism. This activity will be transferred to the National Institutes of Health upon completion of the renovations in the assigned space.

The scientific effort of the Laboratory may be described within three areas:

1) Radiation Biology. For eight years the Columbia Laboratory, in conjunction with the Brookhaven Laboratories, have studied the effect of x-irradiation on the cerebral cortex of the macaca mulatta. The results of the last four years were set forth in Brain Research, 7:1-120, 1968. Subsequent to this publication attention has been directed to the functional and structural changes in the irradiated and non-irradiated cortex over prolonged periods following exposure. A significant finding has been the depression of the evoked response from the homologous non-irradiated cortex without overt structural changes. The mechanism of this "transmitted" influence from the irradiated cortex is currently being investigated with the use of split brain preparations. The results from these investigations should have meaning for the ever expanding human exposure to radiation in both therapeutic and exploratory endeavors.

2) Ontogeny of Focal Seizures. A summary of the previous work in this field is contained in the chapter "Ontogeny of Focal Seizures in the Monkey". In Jasper, H. H., Ward, A. A., Jr., and Pope, A. (Eds.): Basic Mechanisms of the Epilepsies. Boston, Mass., Little, Brown and Co., 1969 (in press). A significant feature of this investigation has been the apparent dependency of the immature cortex on midline thalamic structures in the development of the paroxysmal discharge. This concept is currently being tested by the interruption of the cortico-thalamic circuitry. Begun in the newborn, this procedure will be carried out at successive age levels to demonstrate not only the dependence of the immature cortex on subcortical structures but also the evolving lateral association pathways within the developing cortex. Thus, one should have a clearer insight into the development and propagation of the epileptic discharge in infants, children, and adults.

3) Sleep Studies. The "generators" of the electrical forms of sleep have been sought through surface and depth recording in the monkey. This has begun with observations in the young macaca mulatta (in which the forms are most prominent) prior to the full development and integration of the cortex. The preliminary findings implicate the reticular formation and midline thalamic structures with the deeper phases of sleep and the hippocampus with drowsiness.

In addition to the Laboratory, effort was expended in the organization of the Conference on the Late Effects of Head Injury and the publication of the proceedings: Late Effects of Head Injury. In Walker, A. E., Caveness, W. F., and Critchley, M.(Eds.) Springfield, Ill., Charles C Thomas, 1969 (in press).

Serial No. NDS(I)-69 LEN/OC 1693
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Functional and Structural Alterations Following X-Irradiation of the Cerebral Cortex of the Monkey

Previous Serial Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Arland L. Carsten, Ph.D.
Leon Roizin, M.D.
Perry Black, M.D.

Cooperating Units: Columbia University, College of Physicians and Surgeons, New York
Brookhaven National Laboratories, Upton, Long Island, New York
Johns Hopkins University School of Medicine, Baltimore, Maryland

Man Years

Total:	2.3
Professional:	1.1
Other:	1.2

Project Description:

Objectives: To determine the pathogenesis of early and late effects of irradiation on the central nervous system.

Methods Employed: The right visual cortex, in the young adult macaca mulatta, is exposed to 3500 rads of x-irradiation in a single dose. Functional changes are monitored by evoked electroencephalographic responses from photic stimulation of the retina prior to and at weekly intervals following irradiation. The stimuli are programmed to provide single light flashes that may be averaged and a series of frequencies that range from three to thirty flashes per second in ten second epochs. The evoked response from the irradiated and non-irradiated visual cortex is led through a TV two-channel Grey-Walter, low frequency analyzer that provides histograms for the abundance of activity, or amplitude, for ten seconds at each of the frequencies employed. Thus one can compare

directly the amplitude of the evoked response from the irradiated cortex with that from the homologous non-irradiated cortex.

Sequential anatomical examinations are carried out by sacrificing three animals each at 72 hours, 1, 4, 12, 20, 28, 44, and 144 weeks for routine, Golgi-Cox and electron microscopic preparations.

A selected series will be subjected to corpus callosum section and/or optic chaism section prior to irradiation.

Major Findings: The evoked responses of the visual cortex to photic stimulation has shown a decrease in voltage over the irradiated side. This effect begins soon after exposure, achieves an appreciable degree between 6 and 12 weeks, recedes between 18 and 22 weeks, and is most pronounced beyond 28 weeks. There is some fluctuation in the amplitude from the homologous non-irradiated visual cortex but beyond 90 weeks there has been reduction in amplitude comparable to that of the irradiated side.

An early impairment, within 72 hours, in glycogen metabolism and protein breakdown is suggested from electron microscopy. Routine histology reveals no significant changes in the irradiated cortex, other than mild inflammatory or glial reactions, until around the twentieth week when the beginning of destructive processes is apparent. At and beyond 28 weeks there is severe disorganization of cyto and myeloarchitecture, accompanied by proliferative and degenerative changes in glial and vascular elements. At 4 weeks, well in advance of the described "radiation necrosis", the Golgi-Cox preparations show a significant reduction in the dendritic plexus of the pyramidal cells. This is more evident at 12 weeks with a greater reduction in basilar than apical branches, accompanied by alteration in the pedunculated bulbs. Cell loss is evident from this time, and beyond 20 weeks there is obvious alteration and loss in cells with various degrees of distortion in the remaining neuropil.

The first reduction in voltage of the evoked response from the irradiated cortex is evidently related to the reduction in the dendritic plexus with its concomitant loss in synaptic surface. The more pronounced decrease beyond 20 weeks is related to an estimated dendritic loss of 60% and a cell loss of 30%. The pronounced decrease in evoked response from the non-irradiated cortex beyond 90 weeks is as yet unexplained.

Significance to Bio-Medical Research and the Program of the Institute: The results from these investigations should have meaning and practical prognostic significance in the increasing human exposure to radiation in both therapeutic efforts and space exploration.

Proposed Course: The earliest detectable changes in the routine histology and Golgi-Cox preparations is a reduction in basilar dendrites and their spines. It is reasonable to expect that this will be reflected by the alteration in some part of the wave earlier than a

reduction in the overall amplitude of the response. In short, this should provide an earlier and more sensitive indication of functional change in the irradiated cortex.

An accurate appraisal of the wave form of the evoked responses will be sought through an appropriate instrument computer system. Motohiro Kato, M.D., a visiting scientist skilled in the techniques of such systems, will join the laboratory this year.

A late finding of the irradiation effect is a marked depression in amplitude of the evoked response in the non-irradiated as well as the irradiated side. The instrument system will provide exact timing as well as wave form for the evoked response on each side which should be helpful in determining the mode of transmission of the irradiation effect. Split brain preparations will afford additional insight into the mode of transmission from the irradiated to non-irradiated cortex.

The preceding should provide a better definition of the subtle and profound changes following x-irradiation. A search for the basic mechanism responsible for the early impairment in synaptic function and structure will continue.

Honors and Awards: None

Publications:

Caveness, W. F., Carsten, A. L., Roizin, L., and Schade, J. P.: Pathogenesis of x-irradiation effects in the monkey cerebral cortex. Special Issue, Brain Research, 7:1-120, 1968.

Serial No. NDS(I)-69 LEN/OC 1694
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ontogeny of Focal Seizures

Previous Serial Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Paul I. Yakovlev, M.D.
Raymond D. Adams, M.D.
Francis A. Echlin, M.D.

Cooperating Units: Columbia University, College of Physicians and Surgeons, New York
Harvard University Medical School, Boston, Massachusetts
Lenox Hill Hospital, New York

Man Years

Total:	2.0
Professional:	.8
Other:	1.2

Project Description:

Objectives: To better understand the evolving seizure pattern in the developing brain.

Methods Employed: Focal seizures are induced by injecting 25,000 units of penicillin in 0.025 cc of aqueous solution into the hand-face area of the motor cortex of the macaca mulatta at successive age levels.

The onset and elaboration of the attack pattern is simultaneously recorded by motion picture photography, electromyography, and electroencephalography.

The cerebra of the experimental, and a control series of animals, are examined for the level of development in cells, neuropil and myelin with attention to the motor cortex and subcortical tracts that were deemed relevant.

Major Findings: A significant feature of this investigation has been the apparent dependency of the immature cortex on midline thalamic structures in the development of the paroxysmal discharge. This concept is currently being tested by the interruption of the cortico-thalamic circuitry. Begun in the newborn, this procedure will be carried out at successive age levels to demonstrate not only the dependence of the immature cortex on subcortical structures but also the evolving lateral association pathways within the developing cortex.

Significance to Bio-Medical Research and the Program of the Institute: The results from this investigation should improve the understanding of the age dependent seizure patterns in humans.

Proposed Course: Continuation of the study of the relative significance of cortex and subcortical structures at successive age levels in the development and propagation of the seizure discharge.

Honors and Awards: None

Publications:

Caveness, W. F.: Ontogeny of focal seizures in the monkey. In Jasper, H. H., Ward, A. A., Jr., and Pope, A. (Eds.): Basic Mechanisms of the Epilepsies. Boston, Mass., Little, Brown and Co., 1969 (in press).

Serial No. NDS(I)-69 LEN/OC 1695
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cortical and Subcortical Recording of the Electrical Reflection of Sleep

Previous Serial Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators: Jiri Machek, M.D.

Cooperating Units: Columbia University, College of Physicians and Surgeons, New York

Man Years

Total:	1.2
Professional:	.7
Other:	.5

Project Description:

Objectives: A search for the origin of the principal forms that are reflected in the electroencephalogram during various phases of sleep.

Methods Employed: The "generators" of the electrical forms of sleep have been sought through surface and depth recording in the monkey. This has begun with observations in the young macaca mulatta, in which the forms are most prominent, prior to the full development and integration of the cortex.

The cortical recording is obtained from frontal, temporal, and occipital electrodes, bilaterally. The depth recording is from midline thalamic structures, brain stem reticular formation, and the hippocampus. The observations are carried out during two hours of the normal sleeping period, daily for seven days after the electrode implantation.

Major Findings: The preliminary findings implicate the reticular formation and midline thalamic structures with the deeper phases of sleep and the hippocampus with drowsiness.

Significance to Bio-Medical Research and the Program of the Institute: The results should add to the understanding of the physiological parameters of normal sleep and, long range, to the understanding of the precipitating factors in disease processes, e.g. convulsive seizures coincident with sleep.

Proposed Course: Refinement of techniques and extension of observations to puberty.

Honors and Awards: None

Publications: None

Serial No. NDS(I)-69 LEN/OC 1788
1. Laboratory of Experimental Neurology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Conference on the Late Effects of Head Injury

Previous Serial Number: None

Principal Investigator: William F. Caveness, M.D.

Other Investigators: A. Earl Walker, M.D.
Macdonald Critchley, M.D.

Cooperating Units: World Federation of Neurology
World Federation of Neurosurgical Societies

Man Years

Total:	2.0
Professional:	0.5
Other:	1.5

Project Description:

Objectives: To appraise the state of the art and stimulate research in the late effects of head injury.

Methods Employed: Closed and open sessions that included presentations of papers by 47 participants.

Major Findings: A reasonable appraisal of the knowledge and areas of deficiencies concerning the posttraumatic syndrome, posttraumatic epilepsy, rehabilitation, and medico-legal aspects.

Significance to Bio-Medical Research and the Program of the Institute: A contribution to the understanding and management of the late effects of head injury.

Proposed Course: An exchange of ideas with perhaps a stimulus for the further understanding and management of the late effects of head injury.

Honors and Awards: None

Publications:

Walker, A. E., Caveness, W. F., and Critchley, M. (Eds.): Late Effects of Head Injury. Springfield, Ill., Charles C Thomas, 1969 (in press).

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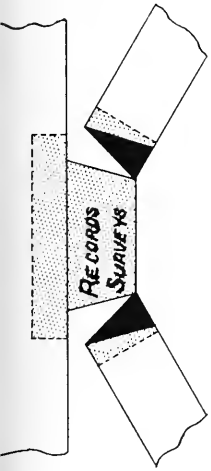

TABBED SEPARATOR SHEET
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ANNUAL REPORT
July 1, 1968 through June 30, 1969
Laboratory of Neurochemistry, Intramural Research
National Institute of Neurological Diseases and Stroke

Dr. Donald B. Tower, Chief

By the criterion of progress among the various research projects of the Laboratory, this past year must be considered a most successful year, despite certain administrative restraints. Many operations were faced with uncertainties over the impending major renovations, to our space in Bldg. 10, which have finally been under way since April 1969. Meanwhile overcrowding in several Sections has reached almost crisis proportions. We shall welcome the relief to be afforded by the modest amount of additional space being provided by the renovations, especially for projects involving tissue culture, protein and enzyme purification and structural analyses, and organic chemical syntheses. The Laboratory is most appreciative of the generosity of a number of other NINDS and NIH units for making space temporarily available to conduct some of our studies elsewhere.

During this past year recognition was afforded each member of the senior staff of the Laboratory for contributions to the field. These took the form of invitations to lecture before distinguished meetings (such as the centenary meeting of the Biochemical Society in England), to contribute reviews or chapters to important monographs, to participate in field studies and scientific expeditions (such as the R/V "Alpha Helix" 1968 expedition), and to assume editorial direction of scientific journals (such as Editor of the Journal of Neurochemistry). Significant collaborations with other NINDS, NIH and outside groups continued. One very fruitful example has been the joint project with the DRS Biomedical Engineering and Instrumentation Branch involving studies in vivo of chloride replacement by extracorporeal dialysis. One of the senior staff has been instrumental in the organization of a Conference on Neurochemistry Curriculum to be held in late June 1969 and aimed primarily at the medical school students. And several of the senior staff have been active in the organizational planning of the future American Society for Neurochemistry.

The individual projects which follow provide details of the satisfying and intriguing progress in each. A few facets deserve to be highlighted here. The studies in depth of the mechanism of action and structural correlates of the Mg-dependent, Na-K-activated ATPase in the Section on Enzyme Chemistry continue to yield fundamental and vital findings. It is clear that the approaches being employed will shortly permit identification of the "active site" of the phosphorylated enzyme and that studies with various inhibitors are providing much insight into the allosteric and conformational aspects of

the mechanisms of action of the enzyme system. Now the addition of complementary studies of a comparative nature should provide further clarification of mechanisms, as e.g. in the rectal salt gland of the shark where only Na-activation is involved. The imaginativeness, ingenuity and diligence of this group of investigators keeps them in the forefront of work in this very important field.

The momentum gained in the past two years has been sustained this year in the Section on Lipid Chemistry along a broad front of investigations. The successful synthesis of labelled Tay-Sachs ganglioside, an achievement in itself, now makes it possible at last to test the hypothesis that it is catabolism of this lipid that is deranged in the disease. This final hurdle in a long and frustrating endeavor should be surmounted very soon. Further delineation of the role of leukocytes in several of the lipidoses and especially the correlations in leukemia continue to prove fruitful, and the extensions to tissue culture, notably with cells obtained by amniocentesis, are already proving of great practical importance both diagnostically and for genetic counselling. A real boost to many of these studies is provided by the new techniques developed in the Section for radioisotope labelling of sphingolipids by an exchange-reaction type of chemical synthesis. And finally the concerted study of myelination and demyelination by techniques of isolating individual species of cells from neural tissues continues to show promise. This is one of the crucial problems currently being attacked by several groups around the world; published methods clearly have two serious shortcomings, lack of clean separation into single cell species and lack of viability and/or integrity of isolated cells. Criteria for assessing the latter aspects have been developed here and are being applied to current preparations, especially with respect to the permeability characteristics of the cells after isolation and their ability to maintain normal intracellular ionic environment and metabolism.

Studies on the membranes themselves continue in both the Section on Physiology and Metabolism, where the role of lipoproteins and phosphoester metabolism in membranes is emphasized, and in the Section on Amino Acids and Electrolytes, where attention is focussed on the highly acidic proteins of the membrane. Both facets relate to mechanisms of transmembrane transport. It is intriguing to find distinctive properties (lower Km values and higher velocities) for membrane-bound enzymes involved in phosphoester metabolism, to find specialized compounds like S-acyl pantetheine in the membrane and lipoprotein species specific for transport of distinctive carotenoid lipids, and to isolate a protein with over 40 percent of its components contributed by glutamyl and aspartyl moieties. The last example is proving most interesting because of its theoretical aspects (shape, properties, and reactivity, of such a highly charged molecule) as well as practical questions of its functional significance.

Unexpected and highly significant findings were provided this year by the Section on Developmental Neurochemistry, together with the Section on Amino Acids and Electrolytes and the Biomedical Engineering and Instrumentation Branch, DRS. Completion of the studies on metabolism of chloride in slices of cerebral tissue incubated in vitro established that one of the cellular compartments (? astroglia) in cerebral cortex takes up chloride by a process of K-dependent, mediated transport and that the extra fluid (edema; swelling) in this compartment is an isotonic expansion with KCl. Thus a correlation with the high-K glial cells reported by others is suggested. The ability to reduce this edema isosmotically by substituting isethionate for chloride led to companion studies in vivo, where extracorporeal dialysis proved effective in achieving controlled replacement of over 90% of the extracellular chloride. Under such conditions chloride in the central nervous system was maintained as much as 11.6 times higher than plasma chloride by virtue of mediated (active) transport from blood to CSF and hence by diffusion into brain. Such transport had been proposed but never actually demonstrated before the present study. These data add significantly to our understanding of the mechanisms responsible for homeostatic regulation of the fluid and ionic environment of neural cells.

Again one is impressed by the unplanned and spontaneous convergence of almost all projects in the Laboratory on the fundamental problems of the functional organization of neural membranes. The steady progress along several fronts: lipids, proteins, enzymes, transport, is most gratifying, since we can now dare to hope that we will soon begin to understand some important facets of such functional organization. It would be presumptuous to expect that many answers will be provided by the projects in this Laboratory, but we can assert with some confidence that at least a few of the important answers will evolve from them.

Serial No. NDS(1)-61 LNC/AAC 810
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Metabolism of Free and Protein-Bound Amino Acids in Neural Tissues.

Previous Serial Number: Same.

Principal Investigator: Drs. D. B. Tower and G. S. Allen.

Other Investigators: Mr. Oscar Young.

Cooperating Units: Section on Enzymes and Cellular Biochemistry, LBM, NIAMD; Laboratory of Neurochemistry, NIMH; Section on Ophthalmology Chemistry, NINDS.

Man Years:

Total:	1.5
Professional:	1.5
Others:	0

Project Description:

Objectives: To investigate the metabolic interrelationships (and factors affecting them) of amino acids, especially of the glutamate and aspartate group, in the free and protein-bound pools of neural tissues, with emphasis on cerebral cortex studied in vitro.

Methods: See under Major Findings.

Major Findings: During the current year efforts have been concentrated on a detailed study of the acidic protein previously demonstrated (see 1964-65 report) in membrane-rich fractions. The previous method of isolation was duplicated: The microsomal fraction of liver was obtained by centrifugal subfractionation and the membrane subfraction was separated from the ribosomal subfraction by solubilization in deoxycholate. The acidic protein was isolated on a DEAE-cellulose column as the final peak to be eluted in 0.5 M phosphate (pH 5.6) containing 0.5 M NaCl. Examination of this peak by electrophoresis on polyacrylamide gel and staining with Coomasie blue indicated a major and two minor components. A variety of subfractionations utilizing various columns achieved somewhat greater purification, but better results have been achieved by lowering the pH of the deoxycholate-soluble microsomal subfraction to 5.6, such that the neutral and less acidic proteins

precipitate. Subsequent separation by column chromatography on DEAE-cellulose (or similar materials) with continuous gradient elution provides a single sharp peak that moves as a single band in polyacrylamide gel. Preliminary analysis by ultracentrifuge of this protein indicates an approximate molecular weight of 45000, and on acid hydrolysis the percentage of glutamyl + aspartyl moieties comprise over 40% of the total amino acid residues. Now that a reproducible preparative procedure has been established, detailed physico-chemical characterizations are in progress and antibodies are being prepared in sheep so that the occurrence and distribution of the protein can be evaluated and in order to assess the effects of phenobarbital (in liver) and Dilantin (in cerebellum) on the amounts of the protein in the newly formed microsomal membranes induced by these drugs.

Significance: This project bears directly upon the fundamental problem of delineating some of the interrelationships between cerebral structure and metabolism, and between metabolism and functional activity, so that an eventual integration of these facets of cellular neurochemistry can be realized.

Proposed Course: To continue present studies along the lines indicated above.

Publications:

None.

Serial No. NDS(1)-61 LNC/AAC 811
1. Neurochemistry
2. Amino Acids & Electrolytes
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electrolytes and Energy Metabolism in Cerebral Cortex in vitro.

Previous Serial Number: Same.

Principal Investigators: Drs. D. B. Tower and R. S. Bourke.

Other Investigators: Mr. Oscar Young.

Cooperating Units: None.

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives: To study the in vitro metabolism of electrolytes and of energy-producing cycles and compounds thereof in incubated slices of cerebral cortex.

Methods: Established methods for incubation of slices of brain tissue in vitro were used with appropriate media and indicator solutes.

Major Findings: During this year work on this project merged temporarily with that in Project Serial No. NDS(1)-68-LNC/DNC 1549 (Bourke: Fluid Dynamics in Cerebral Tissues), while the role and dynamics of chloride metabolism, as described in that project, were being clarified. It had been intended to initiate in the present project the studies on fluxes of Na and K in incubated slices of cerebral cortex (normal and epileptogenic) previously proposed. When it became evident that the role of chloride in slice edema could be characterized and measures for its control specified, the results of these studies have been awaited before embarking on the flux measurements. The latter are enormously complicated in slices of cerebral cortex taken from biopsy specimens removed surgically from patients (or animals) because of the sensitivity of fluid distribution in the tissue to vascular insufficiency during excision. Shifts of fluid intracellularly, especially into the KCl-rich glial compartment (described in Project LNC/DNC-1549), superimpose artifacts on the distributions of fluids and electrolytes which were originally present in the tissue sample before excision. Present renovations underway in the laboratory space of this section have further delayed work on this project, but these delays may

prove advantageous in view of the newly described method of Lewin (Neurology 19: 310, 1969) for preparing experimental epileptogenic foci using a cortical freezing lesion plus topical ouabain. This should provide a suitable alternative to penicillin foci in developing experimental models in which to study cation fluxes.

Significance: Energy-yielding metabolism is the basic factor underlying neuronal function and activity, and electrolyte metabolism (which clearly depends upon it) provides a fundamental link between cellular chemistry and the functional activity of impulse conduction. The understanding of factors involved is essential for the elucidation of both normal functioning of neural tissues as well as deranged functions of hyperactivity states.

Proposed Course: To pursue investigations along lines indicated by previous results.

Publications:

1. Tower, D.B.: Ouabain and the distribution of calcium and magnesium in cerebral tissues in vitro. Exper. Brain Research 6:273-283, October 1968.

2. Tower, D.B. and Tower, D.A.: Heavy water (^2H_2O) as solvent for incubation media in vitro. Comparison of effects on two tissues exhibiting significant water shifts: mammalian cerebral cortex and etiolated pea seedlings. Biochem. Pharmacol., in press.

3. Tower, D.B.: Neurochemical mechanisms in epilepsy. In: Jasper, H.H. (Ed.): Basic Mechanisms of the Epilepsies, Boston, Little, Brown & Co., in press.

Serial No. NDS(1)-68 LNC/DNC 1549
1. Neurochemistry
2. Developmental Neurochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Fluid Dynamics in Cerebral Tissues.

Previous Serial Number: Same.

Principal Investigator: Dr. Robert S. Bourke.

Other Investigators: Mr. Oscar Young, Dr. H. Gabelnick.

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS.

Man Years:

Total:	3.0
Professional:	3.0
Other:	0

Project Description:

Objectives: To study mechanisms of cerebral edema formation and its control.

Methods: The classical techniques of incubation of tissue slices in vitro, utilizing characterized incubation media and indicator solutes were used throughout. Extracorporeal renal dialysis technique used for studies in vivo.

Major Findings: (1) A number of experiments reported previously have shown that (a) formation of cortical edema in vitro is related to the concentration of K^+ in the incubation medium: tissue swelling or edema is a linear function of K^+ concentration in media for the range 27mM K to 125mM K; and (b) the effect of K^+ on swelling is dependent on the presence of Cl^- ion in the medium. (Bourke, R. and Tower, D., J. Neurochem. 13: 1071, 1966). In elucidating further the relationship between K^+ and Cl^- and the swelling of cerebral cortex, the following have been found:

(1) Swelling of cerebral cortex in vitro is a linear function of Cl^- concentration in incubation media over a range 8mM Cl-132mM Cl, with K^+ concentration maintained at $\sim$ 54mM and isosmolarity maintained by replacing chloride with isethionate. In addition, slices incubated for 1 hr in the presence of 54mM K and 132mM Cl in a saline-bicarbonate glucose medium ($\sim$300 mOsm/L) and then transferred to a medium of similar

composition and tonicity but with chloride replaced by isethionate lost ∿80% of the fluid of swelling.

(2) When chloride kinetics were investigated by tracer studies (Cl^{36}) in vitro under the above conditions, it was found that at a constant external [K^+] the rate of entry of chloride into cerebral cortex (after diffusion into the extracellular space) followed saturation kinetics with Vmax = 7.7 μM/g/min and Km = 245. The kinetic data so described differ significantly from a curve describing a simple diffusion ($P = <.02$). Moreover, a clear dependence on [K^+] in incubation media was found. When external chloride was held constant at 6.8 mM and [K^+] varied over a range of 27 to 100 mM, the apparent rate of chloride entry followed saturation kinetics (Vmax = 0.191 μeq/g/min and Km = 30.3. Again upon investigation of the rate of flux of Cl^- under equilibrium conditions, it was found that chloride influx was not statistically different from efflux and that the extracellular space derived by extrapolation was within the mean (±S.D.) determined for influx, a value essentially identical to that previously determined for chloride space in vivo. (Bourke, Greenberg and Tower, Am. J. Physiol. 208: 682, 1965).

(3) Having thus a clear approximation of the extracellular compartment and having thus described the kinetics of fluid and electrolyte loss (in vitro), it was found that the composition of the fluid of swelling was essentially in isotonic expansion in which the predominant ions were K^+ and Cl^-. Moreover, it was apparent that the added swelling was membrane bound and could be reduced isosmotically by (1) lowering external K^+ (Bourke and Tower: J. Neurochem. 13: 1071, 1966) or by replacing external chloride with isethionate. Preliminary toxicity studies in vitro and in vivo indicate minimal, if any, injurious effects resulting from replacement of chloride by isethionate.

(4) Studies in vivo in which plasma Cl^- was isosmotically lowered from 120.5 mM to 8 mM in the adult cat indicate that content and/or concentration of chloride in CSF, cerebral cortex and corpus callosum are relatively resistant to reduction of chloride in the face of reduction of plasma chloride concentration, even when concentration of the plasma chloride is maintained at ∿ 8 mM for several hours.

(5) Flux studies employing Cl^{36} indicate that the mechanism by which CSF chloride is maintained as much as 11.6 times greater than plasma chloride concentrations is clearly by mediated transport (active transport) of chloride from blood to CSF, with a Vmax of 0.5 μM/min and a Km probably below 8 mM.

Significance: Altered fluid dynamics are concomitants of most of the important clinical problems, whether these be hyperactivity, trauma, vascular disorders or tumor, so that an understanding of factors which determine fluid distribution and a delineation of the sites of distribution are essential to rational therapy. These aspects are of particular importance in the developing brain where there are significant changes

in fluid distribution associated with growth and maturation and where there can be frequent superimposition of states of edema, hydrocephalus, and the like.

Proposed Course: To pursue investigations along the lines indicated by present studies, e.g. investigation of the possibility of replacing body chloride by isethionate as a method of combating traumatic cerebral edema (cortical).

Publications:

1. Bourke, R.S.: Evidence for mediated transport of chloride in cat cerebral cortex in vitro. Exp. Brain Res. 8:, in press (1969).

2. Bourke, R.S.: Studies of the development and subsequent reduction of swelling of mammalian cerebral cortex under isosmotic conditions in vitro. Exp. Brain Res. 8:, in press (1969).

Serial No. NDS(1)-61 LNC/EC 813
1. Neurochemistry
2. Enzyme Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Enzymological Aspects of Neural Function.

Previous Serial Number: Same.

Principal Investigator: Dr. R. W. Albers.

Other Investigators: Drs. F. J. Samaha, A. Neims, and Mr. G. J. Koval.

Cooperating Units: Section on Experimental Neurology, LNNS, NINDS.

Man Years:

Total:	5.0
Professional:	4.0
Other:	1.0

Project Description:

Objectives: To assess the functional roles and interrelationships of enzyme systems which characterize neural tissue.

Methods: Isotope studies; classical techniques of enzyme purification and characterization; cytological fractionations.

Major Findings: (1) Several aspects of the Na^+-ATPase system are under study.

(a) We have developed procedures for purifying and solubilizing the "active-site protein" of the Na^+-ATPase of the eel electric organ. These procedures depend upon labelling the active site with radioactive phosphate, which is then used as a marker. Preliminary work indicates that the protein has a molecular weight of the order of 10^5. Brief proteolytic digestion produces a radioactive fragment of M.W.$\sim 10^4$ and fragments of <1000 M.W. can be produced by extensive proteolysis. Considerable progress has been made in the direction of obtaining these small fragments in sufficiently pure form for amino acid analysis and sequencing.

(b) The incorporation of orthophosphate into ouabain treated Na^+-ATPase has been the subject of detailed investigation. We have established that the site labeled by this procedure is identical to that labeled with $AT^{32}P$. This has major theoretical significance for the operation of the sodium transport system. We have measured the equilibrium of both phosphorylation reactions. On the basis of these studies we have constructed a hypothesis for the mechanism of action of cardioactive steroids. The P_i incorporation is considered to occur via the phosphorolysis of an "X-Y" group on the enzyme. Cardioactive steroids promote this reaction by inhibiting the hydrolysis of X-Y, which is the K^+ activated step of the overall reaction.

(c) We have begun an investigation of the NaATPase of shark rectal gland. The shark rectal gland is an organ which is specialized for the excretion of NaCl. This process would seem to require either an electrogenic Na^+ pump or a Na^+-Cl^- coupled pump in contrast to the Na^+-K^+ coupling exhibited by most tissues. A highly active Na^+-dependent ATPase can be prepared from this tissue. Detailed study may indicate some functional modification of the enzyme which would relate it to this type of secretory activity.

(2) Our studies of the differences between red muscle and white muscle myosin ATPases have progressed in collaboration with the Section on Experimental Neurology, LNNS, NINDS.

In addition to the differences in specific activity of red and white myosins, there is a marked difference in pH stability. The two types of myosin can be differentially inactivated by pre-incubation in acid or alkali. Quantitative assays of the relative amounts of the two myosins can be carried out on this basis. The Section on Experimental Neurology will report on the adaptation of this observation to a histochemical demonstration of the distribution of red and white myosins in muscle fibers.

Red and white myosins are further distinguished by their association with modifier proteins. The usual preparations of myosin contain considerable amounts of tropomyosin and troponin. This appears to be true of both red and white myosins. However, white myosin preparations contain two additional proteins which are not found in red myosin and which are only dissociated from myosin in the presence of certain sulfhydroxyl blocking reagents. These proteins seem to be distinct from previously characterized components of the contractile system.

Significance: The studies on Na^+-ATPase contribute to an understanding of the generation of the "resting" membrane potential of cells by active sodium-ion transport. It seems already evident that "resting potential" is a misleading term in that the potential so-designated is results from a balance of dynamic processes. Several workers have recently found

evidence that active Na^+ transport may underlie such diverse phenomena as the temperature sensitivity of the "resting" potential of giant neurons of Aplysia (D. Carpenter, LNP-NIMH), and the photoreceptor mechanism of Limulus (T. Smith, LNLC-NINDS).

The mechanism of action of cardiac glycosides has intrinsic interest as an example of drug-receptor interaction involving a major class of therapeutic agents.

The fact that the NaATPase is an integral component of the plasma membrane permits some generalization of the properties of this enzyme system to properties of other membrane processes: other transport mechanisms, the action potential mechanism, etc.

The extension of our ATPase studies to include myosin ATPase grows logically from several interests of the section which coincide with those of EN-LNNS. There is obvious utility in studying, on a comparative basis, the molecular mechanism of two different ATPase systems, both involved in energy transduction.

In addition the type of myosin in a given muscle cell is under control of the motor nerve fiber. The methods which we are developing are directed toward exploration of this problem. Aside from the clinical importance of such studies to the muscular dystrophies, the nerve-muscle relationship would seem to constitute a simple, experimentally accessible phenomenon which could be analogous to neuron-neuron relationships involving long-term modifications in the central nervous system.

Proposed Course: (1) A major effort will continue to be the chemical characterization of the phosphorylation site of NaATPase. (2) We shall attempt to design experiments to test our hypothesis for the mechanism of action of cardioactive steroids. (3) Comparative studies of NaATPase from other sources, such as the shark rectal gland, will continue. (4) The modifier proteins associated with white myosin will be examined with regard to their role in the contractile process. The techniques developed in this study will be applied, in collaboration with EN/LNNS, to studies of the neural regulation of myosin synthesis.

Publications:

1. Albers, R.W. and Siegel, G.J.: "Nucleotide phosphohydrolases," chapter in "Handbook of Neurochemistry," Ed. A. Lajtha, Plenum Press (in press).

2. Siegel, G.J., Koval, G.J. and Albers, R.W.: Sodium-Potassium-activated Adenosine Triphosphatases. VI. Characterization of the phosphoprotein formed from orthophosphate in the presence of ouabain. J. Biol. Chem. (in press).

Serial No. NDS(1)-61 LNC/LC 815
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Metabolism of Complex Lipids of Nervous Tissue. Studies on Gaucher's Disease, Niemann-Pick Disease, Fabry's Disease, and Metachromatic Leukodystrophy.

Previous Serial Number: Same.

Principal Investigator: Dr. Roscoe O. Brady.

Other Investigators: Drs. A. E. Gal, N. J. Weinreb, and Mr. R. M. Bradley.

Cooperating Units: Weizmann Institute of Science, Rehovoth, Israel; and Division of Biologics Standards.

Man Years:

Total:	2.1
Professional:	1.8
Other:	0.3

Project Description:

Objectives: (1) To elucidate the biosynthetic pathways for the formation of long chain fatty acids, cerebrosides, gangliosides, and sphingomyelin; (2) to study the control mechanisms which regulate these processes; and (3) to study the metabolic fate of sphingolipids in normal and lipodystrophic disease states.

Methods: Glucocerebroside and galactocerebroside labeled with ^{14}C in either the hexose or fatty acid portion of the molecule have been synthesized. ^{14}C-labeled sphingomyelin and gluco-and galactopsychosine have been similarly prepared. Ceramide-trihexoside and ceramide tetrahexoside (globoside) uniformly labeled with radioactive hydrogen-^{3}H have been prepared. The metabolism of these labeled materials has been investigated *in vivo* and *in vitro*.

Major Findings: The enzymes which catalyze the hydrolysis of glucocerebroside and sphingomyelin were demonstrated to be markedly diminished in tissues of patients with Gaucher's disease and Niemann-Pick disease respectively. These findings were extended to enzymatic investigations on washed leukocyte preparations from small samples of venous blood. These latter experiments led to the development of novel and reliable tests for the diagnosis of Gaucher's disease and Niemann-Pick disease.

These studies have been extended to an investigation of the activity of various sphingolipid hydrolases in cells grown in tissue culture. The enzymes involved in Gaucher's disease (glucocerebrosidase) and Niemann-Pick disease (sphingomyelinase) are both very active in cultures of skin fibroblasts and fetal cells obtained by amniocentesis from normal pregnancies. The activity of these enzymes is markedly decreased in skin fibroblast preparations derived respectively from patients with Gaucher's disease and Niemann-Pick disease. The results of the experiments on Niemann-Pick disease have recently been reported (Publications item 4).

In another study carried out during the past year, we have made very satisfactory progress in the purification of the glucocerebrosidase involved in Gaucher's disease. This enzyme is bound to subcellular particles in vivo and has been extremely refractory to more than a moderate degree of purification. We have obtained a completely water-soluble preparation and glucocerebrosidase has now been highly purified by adsorption and differential elution from cationic cellulose columns.

Significance: Our demonstration that the metabolic defect in Gaucher's disease and Niemann-Pick disease persists in cells grown in tissue culture provides another extremely useful diagnostic aid for the detection and differential diagnosis of these diseases. Preliminary studies indicate that extension of this technique may be useful for identifying heterozygous carriers of these abnormal traits. Such a test would be extremely useful for genetic counseling.

In view of the finding of good glucocerebrosidase activity in tissue cultures of fetal cells obtained by amniocentesis, it seems likely that this disease may be amenable to detection in utero. An accurate antenatal diagnostic test for this disease is of immense importance. It is likely that such a test can be developed and performed reliably and sufficiently early in pregnancy that the prospective parents may decide for themselves the desirability of continuing the particular pregnancy to term.

Proposed Course: Attempts will be made to develop accurate, specific tests for detection of heterozygous carriers of sphingolipid storage diseases and to identify the occurrence of afflicted fetuses in utero.

We wish to examine in even greater detail the explicit nature of the enzymatic changes in sphingolipidoses. In order to accomplish this objective, we are attempting to completely purify the enzyme glucocerebrosidase which is involved in Gaucher's disease. We need to know if there is an abnormality in the amino acid sequence of this enzyme in patients with Gaucher's disease. We have already observed significant differences in the substrate affinity and heat-stability of this enzyme obtained from Gaucher patients compared with enzyme preparations from normal human tissue sources. These findings indicate that structural changes have occurred in the enzyme molecule in these patients. Knowledge of the exact nature of these aberrations will be helpful for devising procedures to treat these diseases.

Publications:

1. Brady, R.O.: The metabolism of sphingolipids. In Bolis, L., and Pethica, B.A., (Eds.): Membrane Models and the Formation of Biological Membranes. pp. 114-121. Amsterdam, The Netherlands, North-Holland Publishing Co., July, 1968.

2. Percy, A.K., and Brady, R.O.: Metachromatic leukodystrophy: Diagnosis with venous blood samples. Science, 161: 594-595, August, 1968.

3. Brady, R.O.: Enzymatic defects in the sphingolipidoses. In Bodansky, O., and Stewart, C.P., (Eds.): Advances in Clinical Chemistry, Vol. 11, p. 1-19. New York, New York, Academic Press, Inc., December, 1968.

4. Sloan, H.R., Uhlendorf, B.W., Kanfer, J.N., Brady, R.O., and Fredrickson, D.S.: Deficiency of sphingomyelin-cleaving enzyme activity in tissue cultures derived from patients with Niemann-Pick disease. Biochem. Biophys. Res. Commun. 34: 582-588, March, 1969.

5. Brady, R.O.: Disorders of cerebrosides and disorders of sphingomyelin. In Shy, G.M., Goldenson, E.S., and Appel, S.M. (Eds.): The Cellular and Molecular Basis of Neurologic Diseases. Philadelphia, Pa., Lea and Febiger, in press.

6. Brady, R.O.: Disorders of sphingolipid metabolism. In Ehrenpres, S. and Solnitzky, O. (Eds.): Neurosciences Research Vol. II. New York, New York, Academic Press, in press.

7. Brady, R.O.: The sphingolipodystrophies. In Bondy, P.K., (Ed.): Duncan's Diseases of Metabolism, VI Edition, Philadelphia, Pa. W. B. Saunders, Co., in press.

8. Brady, R.O.: Lipidoses. In Lajtha, A., (Ed.): Handbook of Neurochemistry, Vol. III, New York, New York, Plenum Publishing Corp., in press.

9. Brady, R.O.: Genetics and the sphingolipidoses. Medical Clinics of North America, in press.

10. Brady, R.O.: Cerebral lipidoses. Annual Review of Medicine, in press.

11. Kanfer, J.N., Brady, R.O.: Rat liver sphingomyelinase. In Colowick, S.P. and Kaplan, N.O. (Eds.): Methods in Enzymology New York, New York, Academic Press, Inc., in press.

12. Kanfer, J.N., Blume, R.S., Yankee, R.A., and Wolff, S.M.: Alteration of sphingolipid metabolism in leukocytes from patients with Chediak-Higashi syndrome. New England J. Med. 279: 410-413, August, 1968.

13. Kanfer, J.N.: Incorporation of ^{14}C-UDP-glucose and ^{14}C-UDP-galactose into carbohydrate-containing sphingolipids by a rat brain particulate fraction. Lipids 4: 163-165, 1969.

14. Snyder, R.A., and Brady, R.O.: The use of white cells as a source of diagnostic material for lipid storage diseases. Clinical Chemistry, in press.

Serial No. NDS(1)-61 LNC/LC 816
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Structural and Metabolic Studies of Gangliosides in Normal Humans and Patients with Tay-Sachs Disease.

Previous Serial Number: Same.

Principal Investigator: Dr. E. H. Kolodny.

Other Investigators: Drs. R. O. Brady, A. E. Gal, and Miss J. Quirk.

Cooperating Units: Laboratory of Viral Immunology, DBS; Dept. of Obstetrics, George Washington University Hospital.

Man Years:

Total:	2.3
Professional:	1.3
Other:	1.0

Project Description:

Objectives: To investigate the structure and metabolism of gangliosides.

Methods: Tay-Sachs ganglioside (N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosyl-glucosylceramide) has been labeled in the N-acetylneuraminic acid portion of the molecule with radiohydrogen-^{3}H and in the N-acetylgalactosaminyl moiety with radiocarbon-^{14}C. Labeled precursors of these materials were administered to neonatal rats by intracranial injection. Mixed radioactive gangliosides were isolated from the brains of these animals and treated with bacterial neuraminidase. The product of this reaction, monosialoganglioside, was isolated by preparative thin-layer chromatography. This compound was converted to labeled Tay-Sachs ganglioside through the use of a newly discovered β-galactosidase.

Major Findings: The successful preparation of labeled Tay-Sachs ganglioside has enabled us to demonstrate for the first time the existence of an enzyme in human brain, kidney, heart and intestine which catalyzes the hydrolytic cleavage of N-acetylneuraminic acid from Tay-Sachs ganglioside. Previously described mammalian and bacterial neuraminidases are unable to catalyze this reaction. The products of the reaction have been shown to be N-acetylneuraminic acid and the asialo-derivative of the

parent compound (Tay-Sachs ganglioside minus N-acetylneuraminic acid). These data were reported at the April 1969 annual meeting of the American Society of Biological Chemistry (Atlantic City).

A new diagnostic procedure has been developed in this laboratory for the detection of patients with Tay-Sachs disease using tissue culture preparations of skin fibroblasts. The test is based on quantitative analytical procedures for measuring ganglioside metabolism in tissue culture.

Significance: The preparation of chromatographically pure, labeled Tay-Sachs ganglioside, although admittedly in a very low yield, now permits studies to try to elucidate the nature of the metabolic defect in Tay-Sachs disease.

The development of the specific diagnostic test for Tay-Sachs disease provides an extremely important adjunct to the pediatrician's armamentarium and should facilitate the very early detection of infants with this disease.

Proposed Course: We shall extend our metabolic studies with Tay-Sachs ganglioside using tissue preparations from control human sources and from patients with Tay-Sachs disease in order to try to identify the biochemical defect in this disease.

The tissue culture procedures which we have developed for the diagnostic test for Tay-Sachs disease will be extended with the hope that appropriate modifications will permit the antenatal detection of Tay-Sachs disease. Analyses will be carried out on cultured fetal cells obtained by amniocentesis. Other experiments will be directed towards the potential use of this or a suitably modified protocol for detecting heterozygous carriers of the Tay-Sachs trait. Successful accomplishment of these procedures will be of immense practical value for genetic counseling of families in which there is a history of Tay-Sachs disease.

Publications:

None.

Serial No. NDS(1)-61 LNC/LC 817
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Immunochemical Studies in Multiple Sclerosis.

Previous Serial Number: Same.

Principal Investigator: Dr. R. O. Brady.

Other Investigators: Dr. N. J. Weinreb.

Cooperating Units: National Cancer Institute; Laboratory of Neurophysiology, NINDS; Instituto de Anatomia General y Embriologia, Buenos Aires, Argentina; Kuakini Research Foundation, Honolulu, Hawaii.

Man Years:

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description:

Objectives: To determine whether auto-immune phenomena participate in the pathogenesis of neurological diseases.

Methods: Immunological examinations were made on sera from patients with multiple sclerosis and animals with experimental allergic or viral encephalomyelitis for the presence of antibodies to gangliosides. Animals were immunized with various ganglioside preparations by several procedures.

Major Findings: Anti-ganglioside antibody preparations exhibited an inconstant inhibitory effect on synaptic impulse transmission. When this inhibition occurred, it was reversible and was unaffected by the addition of complement. The results obtained in these studies indicate that preparations with the highest titers of anti-ganglioside antibody which we were able to induce (1:32-1:64) did not block the receptor site. Anti-synaptic membrane protein antibody preparations with a titre of 1:400 effectively block synaptic conduction.

Significance: Successful use of the immunological probe described in this report may provide information related to the chemistry of the receptor site on the post-synaptic neuron. Insight may be obtained in

the possible participation of auto-immune phenomena in certain neurological diseases in the course of studies along these lines.

Proposed Course: Additional attempts are underway to try to obtain anti-ganglioside antibody preparations of high titer. Gangliosides are haptens, and even when they have been injected in combination with various proteins and Freund's adjuvant, only relatively low antibody titers have been produced in immunized animsls. If we cannot obtain antisera with a titer roughly in the range which was shown to be required in the synaptic membrane protein experiments, the experiments will be discontinued.

Publications:

None.

Serial No. NDS(1)-66 LNC/LC 1309
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on the Metabolism of Sphingolipids in Tumor Tissues.

Previous Serial Number: Same.

Principal Investigator: Drs. R. O. Brady and A. E. Gal.

Other Investigators: Mr. R. M. Bradley.

Cooperating Units: National Cancer Institute; and College of Physicians & Surgeons, New York.

Man Years:

Total:	0.7
Professional:	0.7
Other:	0

Project Description:

Objectives: To determine the metabolic pathways of sphingolipids in neoplastic tissues.

Methods: Various complex sphingolipids are synthesized with ^{14}C labels. The metabolic fate of these materials is studied in vivo and in vitro in normal and neoplastic tissue preparations.

Major Findings: The quantity of sphingolipids and the activity of enzymes which catalyze their hydrolysis were determined in spleen tissue obtained from control individuals and patients with chronic myelogenous leukemia. A marked increase in the content of glucocerebroside was found in the spleens of patients with myelogenous leukemia. This observation prompted an investigation of the normal major source of glucocerebroside in peripheral tissues. Our experiments have shown that glucocerebroside arises predominantly from the catabolism of senescent leukocytes and not from senescent erythrocytes as postulated by others. These studies elucidated the etiology of the "Gaucher cells" which are frequently observed in spleens of patients with chronic myelogenous leukemia.

We have pursued other studies on the metabolism of sphingolipids in tissue cultures using primary cell cultures and cell lines obtained by transformation of cells with oncogenic viruses. Our observations indicate that there are significant differences in the content and pattern of sphingolipids in the transformed cells.

Significance: Taken together, these experiments indicate that there are fundamental changes in the metabolism and composition of sphingolipids in transformed and neoplastic cells when compared with normal cells. These findings are of considerable relevance to the possible role played by these membrane lipids in the loss of contact inhibition exhibited by these cells in culture. Elucidation of the control processes which bring about these metabolic alterations is of considerable potential interest for obtaining insight into metabolic changes that occur in oncogenesis.

Proposed Course: The pattern and content of enzymes which catalyze the synthesis and catabolism of sphingolipids will be investigated in tissue cultures derived from normal and neoplastic tissue sources. Parallel studies will be carried out in cells which have been transformed by a number of agents as well as by treatment with oncogenic viruses.

Publications:

1. Kattlove, H.E., Williams, J.C., Gaynor, E., Spivack, M., Bradley, R.M., and Brady, R.O.: Gaucher cells in chronic myelocytic leukemia: An acquired abnormality. Blood, XXXIII, No. 2, Part 2: 379-389, Feb., 1969.

Serial No. NDS(1)-67 LNC/LC 1457
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Chemical Synthesis of Radioactive Sphingolipids.

Previous Serial Number: Same.

Principal Investigator: Dr. A. E. Gal.

Other Investigators: Mr. F. J. Fash.

Cooperating Units: None.

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description:

Objectives: To label sphingolipids with radioactive isotopes of carbon or hydrogen for metabolic studies in hereditary lipid storage diseases and neoplastic conditions.

Methods: Work was done in two directions: specific and non-specific labeling of sphingolipids. As an example of non-specific labeling, the labeling of globoside should be mentioned here. Globoside is N-acetyl-galactosaminyl-galactosylgalactosylglucosyl-ceramide. The material was treated with an excess of tritium gas under pressure. The resulting family of radioactive products required the development of complex radiochemical purification procedures. These techniques were applied to labeling globoside preparations obtained from human red blood cells and from human kidney.

Specific labeling of sphingolipids by chemical procedures would be a major breakthrough of great importance. It would allow the labeling of only one part of a complex lipid molecule and one could then study its function biologically and medically. The major difficulty in this field is that the chemical synthesis of the more complex aminosugar- or neuraminic acid-containing lipids is not known. Nevertheless in the frame of this project, work was done toward the specific synthesis of these complex sphingolipids. A major portion of the work summarized in this report was devoted to the specific labeling of sphingolipids. A new approach has been devised which is based on the exchange of an (inactive) functional group by a ^{14}C-labeled identical group in a sphingolipid

isolated from natural sources. Studies of such exchange reactions were conducted on aminosugars used as models. The results were very promising and it was possible to prepare radioactive sugars by this technique. In order to improve identification of these molecules their behavior in thin-layer chromatographic systems and their spectra in far-infrared was studied.

Major Findings: New preparative radiochemical methods were devised and successfully used for the non-specific labeling of globoside obtained from two different tissue sources. Metabolic studies with these labeled materials indicated that they were two distinctly different compounds. They probably vary in the configuration of the anomeric carbon involved in the glycosidic linkage.

Significant progress was made toward specific labeling of sphingoglycolipids. It is now possible to exchange functional groups of the aminosugar portions of the molecule and introduce radiocarbon labelled groups. The use of these radioactive products should permit detection of metabolic defects in lipid storage diseases in which the nature of biochemical lesion is as yet undisclosed. These labeled chemicals are also of great potential usefulness for developing novel diagnostic procedures.

Significance: Studies on hereditary lipid storage diseases in this laboratory proved that the synthesized labelled sphingolipids are a major tool for studying these diseases. Radioactive materials become more and more important for use in newly developed procedures for the diagnosis of these conditions. It is hoped that in the future the heterozygous carriers of these deficiency diseases can be detected. Once the labeled compounds have been synthesized the tests are relatively easy to perform. It is very likely that procedures for the prenatal diagnosis of many of these diseases will become feasible by extension of these radiochemical labelling techniques.

Proposed Course: Both specific and non-specific labeling of sphingolipids will be studied further. The new approach of labelling with ^{14}C in functional, biologically non-exchangeable groups will be studied on building blocks of glycolipids such as aminosugars and neuraminic acid. Ultimately the method will be used for the specific labeling of glycolipids such as Tay-Sachs ganglioside. Studies toward the eventual total synthesis of gangliosides will also be pursued. Synthetic products can generally be produced in larger quantities and with higher specific radioactivity than that obtainable with materials of biological origin. A workable synthetic approach would provide for extensive use of labelled sphingolipids in diagnostic procedures. These compounds would be made available to clinical chemical laboratories throughout the world.

Publications:

1. Gal, A.E.: Separation and identification of monosaccharides from biological materials by thin-layer chromatography. Anal. Biochem., 24: 452-461, 1968.

2. Gal, A.E., and Fash, F.J.: Far infrared spectra of monosaccharides related to glycolipids. J. Chem. Eng. Data, in press.

Serial No. NDS(1)-68 LNC/LC 1550
1. Neurochemistry
2. Lipid Chemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on Myelination in the Central Nervous System.

Previous Serial Number: Same.

Principal Investigators: Drs. R. O. Brady, R. A. Snyder, R. H. Quarles, S. Max, and R. Sammeck.

Other Investigators: Mr. R. M. Bradley.

Cooperating Units: Yale University School of Medicine; Laboratory of Neurophysiology, NINDS; Laboratory of Clinical Sciences, NIMH; Surgical Neurology Branch, NINDS.

Man Years:

Total:	5.0
Professional:	4.5
Other:	0.5

Project Description:

Objectives: To investigate biochemical events related to the formation and maintenance of the myelin sheath of nerves. In particular, to establish the exact enzymological reactions which occur during the initiation of myelin deposition, to determine how the integrity of the myelin sheath is maintained, and to obtain an understanding of the biochemical pathology which occurs in demyelinating and dysmyelinating lesions in the nervous system.

Methods: Procedures will be developed for obtaining individual cell types from mammalian cerebral cortices. The chemistry and metabolism of these specific cell populations will be investigated using analytical techniques specially developed in this laboratory. Particular attention will be devoted to the biochemical events which occur in the various cells at the time of myelination.

Studies have been undertaken on the effect of denervation of muscles on the chemical composition of neuromuscular end plates. Other investigations have been initiated which deal with chemical and enzymatic changes in demyelinating and remyelinating fiber tracts adjacent to the lateral ventricles in monkeys. Experiments are also underway which are related to the sequence of events involved in the biosynthesis of the basic protein of myelin and the metabolism of glycoproteins at the time of myelination.

Major Findings: We have been partially successful in devising a procedure for the preparation of viable homogenous cell populations from brain tissue. These cells are now being analyzed for their individual structural and metabolic characteristics.

In other studies, we have observed specific changes in the activity of several sphingolipid hydrolases in brain over the life span of rats. Since sphingolipids are major components of myelin, we feel that these changes in metabolic activity are in fact related to the biochemical events which occur during myelinogenesis. The time course of synthesis of the basic protein of myelin has also been demonstrated. Specific enzymes involved in glycoprotein metabolism have been identified in brain for the first time. New techniques have been developed for more accurate quantitation of brain glycoproteins than is possible with the procedures heretofore reported in the literature. Our investigations on the muscle preparations have indicated that these tissues undergo certain characteristic changes in their ganglioside pattern following denervation. The studies on myelination in the monkey brain preparations have just been initiated.

Significance: The development of a satisfactory method for obtaining individual cell types from mammalian brain tissue permits us to undertake critical experiments on the chemistry of the individual species of cells in brain. This procedure should permit us to examine the role and contribution of various types of cells to the formation and maintenance of the myelin sheath. This information is of particular importance for investigating biochemical abnormalities in myelinopathic conditions such as multiple sclerosis and hereditary demyelinating diseases. The studies with denervated muscle preparations may provide insight into biochemical changes in conditions such as cerebral palsy and muscular dystrophy. Investigations dealing with myelin basic protein formation and research on glycoprotein metabolism are relevant to our attempts to understand the processes which occur in the formation of the lipoprotein complexes of the myelin sheath.

Proposed Course: Various analytical and metabolic studies will be undertaken with individual cell species isolated from brain through the use of rate zonal centrifugation. All of the additional procedures and techniques outlined in this report have as their primary goal increasing our knowledge of the biochemical events associated with myelination. It is anticipated that information obtained in the course of these studies will point the way to relevant studies of neuropathies in humans.

Publications:

1. Radin, N.S., Hof, L., Bradley, R.M., and Brady, R.O.: Ceramide lactoside galactosidase: Comparison with other sphingolipid hydrolases in developing rat brain. Brain Research, in press.

Serial No. NDS(1)-65 LNC/PM 1242
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Mechanism of Lipoprotein Synthesis.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Dr. J. Skidmore, and Mr. C. J. Lauter.

Cooperating Units: None.

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To investigate the mechanism of lipoprotein synthesis as a potential model for the assembly of lipid-protein interphases in membranes.

Methods: Lipoprotein synthesis was studied in the rat by using isotopic precursors for the protein and lipid moieties of the complex lipoprotein particle. The plasma lipoproteins were obtained by floatation in the ultracentrifuge at different densities. The incorporation of free fatty acids (FFA) into the lipoprotein lipids was measured after chromatographic separation of the major lipid classes. The delipidized lipoproteins were subjected to acid hydrolysis and their amino acid composition was determined. Incorporation rates of various amino acids into the protein were measured in fasting animals and in animals which were synthesizing lipoproteins actively following a diet load.

Major Findings: The utilization of injected, labeled amino acids for synthesis of plasma proteins increased over a five hour period from 1.35% (1 hour) to 4.13% (6 hours). Since the lipoproteins constitute only about 2% of the total protein mass in the rat plasma, localization of the label in excess of two per cent (4.6 - 6.8%) in these fractions indicated more rapid synthesis (and also faster turnover) of the lipoproteins. This was also reflected in the specific activities of the protein moieties of the lipoproteins. Our data on the changes with time of the specific activity of the different lipoprotein fractions were consistent with turnover rates which had been established for the lipids of the lipoproteins.

Examination of the amino acid composition of the lipoprotein fractions did not reveal substantial differences from those reported previously. There were, however, some changes with time in respect to a few amino acids which were also reflected in changes in specific activity. Composition of the major constituents in the various lipoprotein fractions during the different time periods varied by not more than ten per cent. For instance: (in Mole %; glutamic = 16.9 ± 1.6; aspartic = 11.6 ± 1.2; alanine = 9.0 ± 1.0). The proportion of glycine and serine in the low density fractions (d 1.006 - 1.019) was significantly higher than in the HDL while in the latter we found again a higher percentage of lysine, methionine, histidine, tyrosine and arginine.

When the incorporation patterns of the individual amino acids into the various density fractions were compared, it was concluded that the similarities were considerably more striking than the dissimilarities. Aspartic and glutamic acid were notable exceptions, in that their specific rates of incorporation were lower by several orders of magnitude. This could have been due to rapid metabolic conversion of the isotopic precursors, to preferential uptake by sites other than those concerned with lipoprotein synthesis, or to higher dilutions with endogenous glutamate or aspartate than estimated. In the high density lipoprotein fraction (d 1.21) the average increase of specific activity in the amino acids was 127% during the 1 to 3 hour period. During the subsequent 3-6 hour period another average increase of about 30% was noted in the specific activity. This general trend was not found in the LDL or VLDL where a marked diversity existed in the labeling pattern of individual amino acids. These observations did not reveal a characteristic amino acid composition which might explain a selectivity for particular lipids. It was concluded, however, that the protein moiety of the lipoproteins was primarily synthetized de novo when a metabolic demand occurred for lipoprotein formation.

Significance: This project was developed, because of some of our current thinking about the structure and function of the plasma membrane. It provided us with a useful model. We had postulated that the membrane was composed of a lipid-bilayer with protein covering certain portions of the bilayer and also interdigitating with the lipid phase in some areas. The concept was that a template mechanism or a coding principle exists which will determine the composition of the lipid phase. It was reasoned that a similar, if not identical, problem exists in the formation of the plasma lipoproteins and probably the myelin sheath of nerves. The study of lipoproteins synthesis appeared to represent a logical access to this problem and was selected because a facile experimental approach seemed feasible.

Proposed Course: We propose to continue to search for and interrogate other model systems which may yield information on the assembly of lipid-protein aggregates. Our current investigations with isolated plasma membranes may provide more direct approaches to this problem.

Publications:

1. Trams, E.G.: Carotenoid transport in the plasma of the scarlet ibis (Eudocimus ruber). Comp. Biochem. Physiol., 28, 1177 (1969).

Serial No. NDS(1)-67 LNC/PM 1480
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Metabolism of Neurohumoral Transmitter Substances in Marine Animals.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Dr. D. P. Rall and Dr. E. A. Brown

Cooperating Units: National Cancer Institute and National Heart Institute; Mote Marine Laboratory (Sarasota, Fla.); R/V "Alpha Helix" (Scripps Oceanographic Institute).

Man Years:

Total:	0.15
Professional:	0.15
Other:	0

Project Description:

Objectives: To explore the opportunities offered by the marine environment for the study of comparative neurochemistry. This study in particular represents an attempt to follow the development of neurohumoral transmitter substances and their metabolism in animals lower on the evolutionary scale. Pelagic organisms have developed not only variants of transmitter substances (such as octopamine) but also a variety of potent blocking agents (neurotoxins) and it is hoped that a better understanding of their structure and metabolism may aid in elucidating the modes of function of neurohumoral transmitters.

Methods: During the last year we have installed a small biochemistry laboratory in cooperation with the National Cancer Institute on the grounds of the Mote Marine Laboratory in Sarasota, Fla. This facility is equipped with most of the research tools which are employed in sophisticated biochemical methods. The resources of the Mote Marine Laboratory have been available to investigators which made use of the installation. Our studies, in particular, required the use in vitro of enzyme preparations and of isotopic tracer techniques. Some investigations were conducted aboard the R/V "Alpha Helix" during the 1968 Bering Sea Expedition.

Enzymes which participate in oxidation, reduction, and methylation reactions were studied in vitro, and their characteristics determined by standard techniques. In a number of tissues, partial purification of certain enzymes was routine. The major part of this study was concerned with metabolic reactions in the chondrichthyes, because this class represents a fairly linear evolutionary development extending from late Middle Devonian times.

Major Findings: (1) The interrenal body of the elasmobranch contains significant amounts of the important neurohumoral agents, norepinephrine and epinephrine. It has been shown that this tissue can extract labeled norepinephrine from the circulation efficiently and that it contains the enzymes which provide the relevant major metabolic pathways. The presence of N-methyltransferase indicates, that the interrenal body functions to transform norepinephrine to epinephrine. O-Methylation of the catechols by COMT seems to be the pathway used in preference to monoamine oxidase as the primary reaction for inactivation of the neurohumors.

The metabolism of adrenal medullary hormones has been linked to adrenal cortical steroids. Our data strongly suggest that the conversion of norepinephrine to epinephrine takes place in the adrenal cortical tissue, i.e. interrenal body tissue in the shark. Not much is known at the present time about the innervation of the elasmobranch interrenal body, but it is possible that part of the tissue is composed of nerve endings or that some primitive forms of nerve endings and catechol storage vesicles play a role in the synthesis of transmitter substances. It is suggested that this particular tissue can be utilized to elucidate the interrelationship between corticosteroids and catecholamines.

(2) During the 1968 R/V "Alpha Helix" Bering Sea Expedition, the coastal regions of British Columbia, Canada, provided an opportunity to observe the pink salmon (O. gorbuscha) during the spawning process. Brain cholinesterase was estimated in homogenates using acetylthiocholine as a substrate. Alkaline phosphatase was measured with p-nitrophenyl-phosphate. Catechol-O-methyltransferase (COMT) was determined by the method of Axelrod et al. using S-adenosylmethionine (methyl-^{14}C) as the methyl donor. Catecholamine content was determined fluorometrically.

Cholinesterase of the salmon pituitary underwent a significant decline during the spawning run. The trend, if any, in the other brain portions was also downward. During the same period, the pituitary content of epinephrine and norepinephrine decreased from about 2.76 to 1.37 nanograms per mg fresh tissue. Catechol content of the other brain sections on the average remained fairly constant. Assay of COMT with l-norepinephrine as substrate gave a fairly uniform average activity. Most pronounced was the decrease in alkaline phosphatase activity. This enzyme has been associated with the nuclei of the neurons in the brain but may also play a role in other cellular functions.

Significance: Investigators from several other institutes have made use of the marine facility established by us, and we anticipate that its use for comparative biological studies will increase. Advances, arrests or specializations in the evolutionary process can facilitate the task of the biologist in studying certain complex mechanisms. Some pelagic species, such as the elasmobranchs, have not evolved significantly since Eozoic times. Thus some insight may be gained into the paleobiology of some biochemical processes and their evolution.

Our observations on catecholamine metabolism in elasmobranchii may lead to a better understanding of the role of adrenal cortical steroids in modulating adrenergic transmitter function.

The metabolic collapse of the Pacific salmon has the earmarks of a predestined bioprogram. Because, during many metabolic disturbances, the steady state of the brain appears unperturbed, our observations of changes in brain enzyme levels may point to the origin of metabolic failure in the migratory cycle of this fish. Conceivably, the genetic input of the Pacific salmon dictates an irreversible degeneration of the CNS. The consequence would be rapid aging accompanied by the diverse pathologies evident after "natural" death. The implications for human medicine are obvious.

Proposed Course: We will continue our studies on the role of the cortical steroids and the interrenal body in chondrichthyes.

We shall also attempt to trace the evolution of catecholamine metabolism through some representative species. In addition we propose to examine the role and metabolism of the catechols in the sympathetic nervous system of the elasmobranch.

Publications:

Trams, E.G. Neurochemical observations on spawning pacific salmon. Nature, in press.

E.G. Trams, member 1968 R/V "Alpha Helix" Bering Sea Expedition.

Serial No. NDS(1)-67 LNC/PM 1481
1. Neurochemistry
2. Physiology and Metabolism
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on the Composition and Metabolism of Isolated Cellular Membranes.

Previous Serial Number: Same.

Principal Investigator: Dr. E. G. Trams.

Other Investigators: Dr. J. Skidmore, Dr. J. E. Franklin.

Cooperating Units: None.

Man Years:

Total:	2.65
Professional:	2.65
Other:	0

Project Description:

Objectives: This project is designed to further our understanding of the composition, structure and function of the cell membrane. The primary objective has been to elucidate the relationship of membrane structure to impulse propagation and to the phenomenon of bioelectrogenesis. In the past, however, numerous obstacles have been encountered in attempts to isolate adequate amounts of neuronal membranes, such as giant axon membranes or pure myelin sheath. For this reason, membrane structures of other organs have been examined as methods become available for their preparation. The problem subsequently was approached from two directions: 1) An analytical study of the components of isolated cell membranes and 2) a study of certain functions of the membranes in an effort to isolate the principal molecules participating in these processes.

Methods: Liver plasma membranes were prepared as reported previously. In addition, plasma membranes were isolated from adipose tissue, intestinal mucosa and from electroplax of E. electricus. With radioactive precursors the turnover rates for a number of the component building blocks were estimated in order to establish an index for metabolic activity (or participation in events). As a second approach, the plasma membranes were isolated and synthesis or degradation reactions were followed by using radioactive substrates.

Plasma membranes from rat liver homogenates were characterized by composition and the presence of particular enzymes which have been associated with membrane function. Lipid composition of the membrane was studied in detail and was compared with that reported for other membranes. Palmityl-, stearyl-, oleyl- and linoleyl-CoA esters were prepared enzymatically from radiocarbon labeled acyl precursors. These substrates were incubated with the membranes and the incorporation of isotope into various lipids was investigated. The metabolism of various nucleotides by plasma membranes was studied with a variety of techniques which are currently in use in this field.

Major Findings: Small amounts of neutral glycerides were formed in membranes from rat liver. Unsaturated acyl-CoA derivatives were better utilized precursors than the saturated analogs. Fatty acids were principally incorporated into phosphatidic acid, phosphatidylethanolamine and phosphatidylcholine, presumably through a reacylation of their lyso derivatives.

During incubation of a variety of plasma membrane preparations with S-acyl-CoA esters substantial amounts of a novel lipid were produced. This material was identified as S-acyl pantetheine. Characterization of palmityl pantetheine was achieved by infrared and mass spectrometry, by analysis for its constituent groups, and by chemical synthesis.

Formation of acyl pantetheines was studied in a variety of liver subcellular fractions. Plasma membrane preparations gave by far the highest yield, as did membrane preparations from adipose and electric tissue.

Formation of acyl pantetheine from acyl-CoA was accompanied by P_i liberation and the production of free fatty acid. The reactions leading to the formation of S-acyl pantetheines depend on a membrane nucleotide pyrophosphatase and a phosphomonoesterase activity. These findings led to a more exhaustive investigation of the pathways in which nucleotides are metabolized in the plasma membrane.

In complex nucleotides, such as acyl coenzyme A, or uridine-diphosphoglucose (UDPG) or NADP, the nucleotide is attacked at the pyrophosphate bond, forming a phosphate monoester and a nucleotide residue. In the case of UDPG for instance, these products are glucose-1-phosphate and UMP. The phosphate mono-esters are metabolized by membrane enzymes which are closely related to alkaline phosphatases. In the case of acyl coenzyme A, a very labile enzyme (deacylase) removes the fatty acid by hydrolytic cleavage.

The nucleoside polyphosphate residue, if it can be converted to a 5' nucleotide is rapidly dephosphorylated and converted to a nucleoside. A series of more complex reactions occurs with nucleotide products which contain two or more phosphoryl residues. Some of these reactions are presently under investigation.

At the present time the following enzymatic activities have been found to be associated with one or several of the plasma membrane preparations under study: nucleotide pyrophosphatase, inorganic pyrophosphatase, alkaline phosphatase, nonspecific phosphomonoesterase, 5' nucleotidase, nonspecific phosphodiesterase, specific 3', 5' cyclic nucleotide diesterase, thioester deacylase, nonspecific esterase, and Na^+, K^+ activated ATPase. We have measured for most of these enzymes the reaction velocities, K_m values, pH optima, substrate specificities and the effect of various inhibitors and metal ion requirements.

Our studies on the role of S-acyl pantetheine in transmembrane transport of fatty acid has been inconclusive. Acyl translocation reactions have been demonstrated with liver membranes and with isolated fat cell ghosts but the mechanisms involved are not clear at the present time.

Significance: Our studies have shown that plasma membrane preparations characteristically contain a variety of enzymes which affect phosphoester metabolism. In the case of the ATPases, the reactions undoubtedly are associated with transport of metal ions across the cell membrane. Much less clear is the role of the other enzymes involved in the metabolism of organic phosphates. The activities observed have usually lower K_m values in membranes than in other subcellular particulate fractions and the reaction velocities are high. It is suspected that most of these enzymes are involved in the translocation of acyl-, glycosyl-, or amino acyl residues across the cell membrane.

The studies reported here are directed towards elucidating the molecular concepts which underlie the phenomenon of transport of metabolites across the membrane.

Proposed Course: It is planned to appraise the biological significance of these reactions. It is hoped that the answers for the following questions can be provided by a continuation of our present project:

1. What is the composition of various biomembranes?
2. How is membrane lipid composition regulated?
3. Does the membrane lipid composition influence or determine its function(s)?
4. How do lipids, sugars, amino acids and lipoproteins pass through the plasma membrane? Diffusion? Active Transport? Facilitated diffusion? As Protein Complex? As a high energy phosphate- or thio ester? etc.

Eventually, a concept which encompasses the basic elements of a variety of known transport mechanisms may be formulated.

Publications:

1. W. L. Stahl: The subcellular distribution and nucleotide specificities of (Na^+ and K^+) ATPase and (^{14}C) ADP-ATP exchange reactions in rat brain. J. Neurochem. 15: 499-510 (1968).

2. W. L. Stahl: Sodium stimulated (^{14}C) ADP-ATP exchange activity in brain microsomes. J. Neurochem. 15: 511-518 (1968).

3. Trams, E.G., H. A. Fales and A. E. Gal: S-palmityl pantetheine as an intermediate in the metabolism of palmityl coenzyme A by rat liver plasma membrane preparations. Biochem. Biophys. Res. Comm. 31: 973-976 (1968).

4. Stahl, W. L. and Trams, E.G.: Synthesis of lipids by liver plasma membranes. Incorporation of acyl-coenzyme A derivatives into membrane lipids in vitro. Biochim. Biophys. Acta 163: 459-471 (1968).

5. Trams, E.G., Stahl, W. L., and Robinson, J.: Formation of S-acyl pantetheine from acyl-coenzyme A by plasma membranes. Biochem. Biophys. Acta 163: 472-482 (1968).

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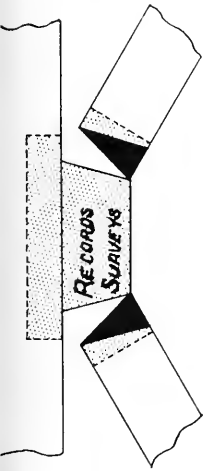

TABBED SEPARATOR SHEET
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(3-56)

ANNUAL REPORT
July 1, 1968 through June 30, 1969
Laboratory of Molecular Biology
National Institute of Neurological Diseases and Stroke
Intramural Research

Ernst Freese, Chief

A. Mutagenic and Inactivating Alterations of DNA

The reaction of chemicals with DNA can induce "mutagenic" and "inactivating" DNA alterations. Mutagenic DNA alterations allow the replication of the altered DNA and induce point mutations. Inactivating DNA alterations are drastic enough to prevent the replication of the altered DNA except when the alteration is repaired by specific enzymes. Compounds that induce inactivating DNA alterations also produce chromosome breaks and induce latent viruses. As a consequence of such alterations, teratogenic effects, cancer, and heritable defects can arise. Certain agents which are either mutagenic themselves or which can be converted into mutagenic agents by cellular enzymes are used as food preservatives, drugs, herbicides, or pesticides. These agents fall into essentially two classes, radical-producing agents and alkylating agents. We have examined the chemical effects of the simplest agents of these two types on oligonucleotides and DNA.

1. The Chemical Effects of Hydrogen Peroxide on Oligodeoxynucleotides.

H_2O_2, which produces OH radicals, reacts mostly with the 5,6 double bond of pyrimidine bases, causing the breakage of the bases and producing inactivating DNA alterations. It also converts adenine to 7-N-hydroxyadenine, which may be responsible for a small point mutagenic effect of radical agents. In addition, H_2O_2 causes the removal of all four bases from DNA, producing unstable deoxyribonic acid which subsequently leads to the breakage of the DNA backbone and thus to chromosome breaks.

2. DNA Backbone Breakage Induced by Alkylating Agents.

Alkylating agents such as methylmethane sulfonate (MMS) and ethylmethane sulfonate (EMS) are known to alkylate mainly guanine and, less frequently, adenine and cytosine; thymine is not affected. Alkylated guanine induces point mutations. In addition, the bonds of the alkylated guanines or adenines to deoxyribose are unstable, such that these bases are slowly removed from DNA at neutral pH; the depurinated sites can eventually lead to DNA backbone breakage. This breakage reaction was assumed to be mainly responsible for the observed decrease in the molecular weight of alkylated DNA. However, our experiments with oligodeoxythymidylic acid have clearly shown that the alkylation of the phosphate groups, producing phosphate triesters, also produces breaks of the sugar phosphate backbone and these breaks occur more readily than those requiring first depurination and then breakage. A short time after DNA treatment, most backbone breakage will therefore result from the phosphate triester breakage, and depurination breakage will become comparable in frequency only

after prolonged incubation for 24 hours or more. These results explain the specificity of DNAases and repair enzymes, which have been found to affect only part of the alkylated DNA damage. A given repair system apparently can eliminate only alkylated bases but not alkylated phosphate triesters.

B. Control Mechanisms and Differentiation

Differentiation in higher organisms depends on many genetic and biochemical mechanisms which are also found in microorganisms, particularly in the process of bacterial sporulation. Since bacteria grow and differentiate faster than higher organisms and since mutants can be easily isolated, the examination of differentiation is greatly facilitated in these organisms. One typically observes during differentiation that DNA synthesis stops and RNA and proteins also increase no longer in amount but they continue to turn over. To understand these control mechanisms the rate and regulation of the synthesis of different types of RNA and of certain proteins are being examined in bacteria. To determine the biochemical pathways required for differentiation, mutants are used which cannot sporulate or are not repressed normally and new techniques have been developed to examine their biochemical blocks. The knowledge of the altered biochemical reactions allow conclusions about the normal process of sporulation and its control by different carbon sources. The attempt to cure such sporulation mutants has shown that the metabolic reactions require a much more delicate control for differentiation than for growth.

1. Control of the Rate of RNA Synthesis.

The rate of RNA synthesis depends on the growth medium and the physiological state of the growing cells. If cells are transferred from a poor to a rich medium, the rate of RNA synthesis increases due to an increase in the frequency at which new chains are produced. This increase involves all three types of RNA--ribosome, transfer, and messenger. When the growth medium is exhausted, the total amount of RNA increases no longer, but all types of RNA continue to turn over. This turnover is possible because all nascent RNA and some ribosomal and transfer RNA are degraded by RNAases and are continuously resynthesized. Organisms can thus adapt to changes in the environment without cellular growth.

2. Regulatory Relationship between RNA and Protein Synthesis.

All 20 amino acids must be present in order to enable a normal rate of RNA synthesis. The lack of any amino acid, in a mutant deficient for it, immediately stops RNA synthesis, except when "relaxed mutants" are used. During the amino acid starvation, the triphosphate precursors of RNA can no longer be made in the stringent strains, but can still be formed in the relaxed mutants. Following starvation of an amino acid, a guanine tetraphosphate has been found to accumulate in the stringent but not the relaxed strain. The exact structure of this compound and its effect on amino acid control are being determined.

3. Control of Dihydrofolate Reductase.

Cellular enzyme synthesis can be controlled by inducers, by repressors, or it can be metabolically stable (constitutive). Whereas induction and repression have been extensively studied, constitutive enzyme synthesis has been neglected. Some constitutive enzymes are made in large amounts, up to 5% of the cellular protein, while others comprise only a few molecules per cell. Dihydrofolate reductase is a constitutive enzyme of the latter type. Its function but not its synthesis can be inhibited by trimethoprim. Trimethoprim-resistant mutants have been isolated which show a 30-fold increase in the specific activity and multiple mutations have been found in which the enzyme activity increased up to 600-fold. In some of these mutations the catalytic site of the enzyme is altered simultaneously, as shown by the change of the pH optimum, the binding of substrate (K_m), or the binding of trimethoprim. Genetic mapping has established that the mutation causing an increase in activity is located at the same or very close to the site that affects the structure of the enzyme; by complementation tests the mutation was shown to be dominant. These results suggest that the structural mutation may also affect the amount of enzyme synthesis. Attempts are now under way to determine whether this is due to a control of messenger RNA or protein synthesis.

4. Biochemical Identification of Sporulation Mutants.

Since most sporulation mutants can grow on glucose minimal medium, some characteristic other than growth has to be used to identify their biochemical lesions. At first, the incorporation of uracil into RNA was used for this purpose, but more recently it was discovered that the measurement of ATP allows an easier and faster characterization. The normal sporulating strain produces ATP both during vegetative growth and during the developmental period. In contrast, most sporulation mutants show during the developmental period a decline of ATP synthesis which can be reversed by the addition of certain carbon sources. The response to different carbon sources allows one to identify the area on the biochemical map in which these mutants are blocked. Dissimilation of radioactive glucose or glutamate and enzyme assays have been used to identify the biochemical lesions in detail.

Some sporulation mutants were found to be blocked in the citric acid cycle. Their response to different carbon sources showed that in the normal strain glucose and other carbon sources are used to produce ATP via the Embden-Meyerhof pathway during the vegetative growth period, whereas ATP is derived from acetyl-CoA via the citric acid cycle during the developmental period. Consequently, mutants lacking pyruvate dehydrogenase were able to grow until they had practically used up all acetyl-CoA-yielding compounds in the medium, but they could not sporulate subsequently unless acetate was added. Interestingly, the acetyl-CoA starvation entailed a complete lack of NADH such that addition of any NADH-producing compound allowed some additional growth, even without addition of acetate. In contrast to other carbon sources, acetate, at concentrations up to 0.08 M, did not repress the onset of sporulation. This explains the importance of acetate for the differentiation of many microorganisms.

Some sporulation mutants are unable to produce an antibiotic activity against Staphyloccocus aureus and conversely, mutants lacking antibiotic activity cannot sporulate. These results suggest that the antibiotic production may be necessary for sporulation. Purified antibiotic activity actually affects Bacillus subtilis itself, because it causes the lysis of cells when added during the exponential growth period.

5. Curing and Repression of Sporulation Mutants.

It was expected that sporulation mutants whose biochemical lesions were known could recover sporulation when the missing compound was added to the medium. However, experimentally this was not found to be true for most mutants. None of our citric acid cycle mutants could be cured with respect to sporulation by the addition of any citric acid cycle compound whether that was added at one time or continuously. This inability was correlated to the lack of ATP during the time during which sporulation should occur. A glucosamine-requiring mutant displayed the dilemma even more clearly. As long as glucosamine was present in the medium, it could be converted to fructose-6-phosphate and thereby give rise to a repressor of sporulation. When glucosamine ran out and sporulation should start, not enough glucosamine was available for a normal sporulation process. Even when small amounts of glucosamine were continuously added to the culture, no normal spores were found. However, the production of abnormal spore-like particles was then observed. In the electron microscope these particles revealed an unusual spore cortex which apparently could not be properly formed because it contains a mucopolypeptide, of which one component is acetyl glucosamine. The delicate control of production and destruction of glucosamine-6-phosphate by the cells apparently cannot be mimicked in the mutants.

Nevertheless, three types of mutants could recover normal sporulation. One was unable to produce its own uracil and stopped growing at the time at which uracil ran out of the medium. Addition of uracil restored both normal growth and sporulation. This shows that deficiency of uracil cannot be the cause of the initiation of sporulation. Similarly, an acetate-requiring mutant stopped growing when acetate had been used up, but it recovered both growth and sporulation when acetate was added to the medium. Also another mutant, whose biochemical lesions have not yet been identified, could recover sporulation in the presence of acetate. This shows that deficiency of acetyl-CoA does not cause the initiation of sporulation.

Other mutants are now under investigation in which sporulation is possible in the presence of excess glucose, which represses the sporulation of normal strains. These mutants are blocked at some step in the metabolism of sugar phosphates. These studies promise to reveal the mechanism by which differentiation is repressed in vegetatively growing cells.

Serial No. NDS(I)-62 LMB/OC 947
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Structure and Alteration of DNA and Chromosomes

Previous Serial Number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Hans-Jürgen Rhaese, Ph.D.

Cooperating Units: None

Man Years

Total:	2.4
Professional:	1.2
Other:	1.2

Project Description:

Objectives: Many chemical agents induce inactivating DNA alterations which in turn give rise to chromosome breaks and the induction of latent viruses. Two major classes of agents can be discerned, radical-producing compounds and alkylating agents. They can both inactivate transforming DNA and the latter can also induce point mutations. The chemical reactions of these two classes of agents with DNA have been examined in detail by mono- and oligonucleotides of DNA.

Methods Employed: Oligodeoxyadenylic acid and oligodeoxythymidylic acid were chemically prepared and purified by column chromatography. Their reactions with hydrogen peroxide and alkylating agents were measured spectrophotometrically, by paper chromatography, by paper electrophoresis, and by radioactive incorporation techniques.

Major Findings: 1. The reaction of hydrogen peroxide with oligodeoxyadenylic acid and DNA nucleotides. H_2O_2 reacts mainly with the pyrimidine bases by opening the 5,6 double bond. It causes the removal of all four bases from DNA or from nucleotides by hydroxylation of the 1 position of deoxyribose. Following the base liberation an unstable deoxyribonic acid is formed which subsequently causes the breakage of the sugar phosphate backbone by β-elimination. In addition, adenine is hydroxylated at the 7-N-position, a reaction which may be responsible for a very small point mutagenic effect of hydrogen peroxide.

2. Backbone breakage by alkylating agents. Alkylating agents are well known to alkylate guanine bases at the 7 position in DNA. This effect induces point mutations and, after prolonged incubation, causes the liberation of the guanine bases from DNA. Since depurinated DNA is labile to mild alkali depurination had been assumed to be the major reaction leading to DNA backbone breakage. However, our investigations on the alkylation of deoxythymidylic acid has shown that the phosphate moiety is alkylated about twice as frequently as the guanine bases and that phosphate triesters can break at the sugar phosphate linkage. A quantitative investigation revealed that the triester breakage occurs with about equal frequency as depurination and both reactions increase with the square of the time of treatment. Since the backbone breakage by depurination increases with the third power of the time, phosphate triester breakage will represent the major cause of backbone breakage after short times of treatment. When the treated DNA is further incubated, both phosphotriesters and depurinated DNA can produce backbone breakage which eventually reaches a comparable frequency. These results explain the biological finding that enzymes, which recognize alkylated purine sites of DNA and cause their repair, are unable to recognize other alkylated sites, i.e. phosphate triester; hence one repair system cannot eliminate all inactivating DNA alterations induced by alkylation.

Proposed Course of Project: Methylation and ethylation of bacteriophages are known to have vastly different effects with respect to induction of point mutations. This phenomenon is not understood. It may be related to repair mechanisms or to the mechanism of mutation induction by alkylating agents. By the use of transforming DNA, the biological and chemical difference between ethylation and methylation will be determined.

Significance to Bio-medical Research and the Program of the Institute: Most neurological diseases are genetically inherited. Some of the mutations responsible for these defects may have been induced, in the parents or during the development of the child, by drugs, pesticides, or other chemicals present in the environment. Such chemicals are alkylating agents, hydroxylamines or hydrazines or they are converted into these compounds by enzymes. The knowledge of the chemical structure of mutagenic chemicals and of their reactions with DNA will help to predict which chemicals are potentially hazardous and should be used with caution.

Honors and Awards: E. Freese, chairman and speaker at a symposium on chemical mutagenesis at the International Congress of Genetics held in Tokyo, Japan, and speaker at the symposium on the history of DNA at the American Chemical Society meeting in San Francisco, 1968.

Publications:

Freese, E.: Hereditary DNA alterations. Angew. Chem. 8: 12-20, 1969.

Freese, E., Rhaese, H. J., and Freese, E. B.: Chemical DNA alterations causing inactivation and chromosome breaks. Japan J. Genet. 44: Suppl. 1. In press.

Rhaese, H. J. and Freese, E.: Chemical analysis of DNA alterations. IV. Reactions with monofunctional alkylating agents leading to backbone breakage. Biochim. Biophys. Acta. In press.

Serial No. NDS(I)-65 LMB/OC 1208
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Ribonucleic Acid and the Regulation of Cellular Metabolism.

Previous Serial Number: SAME

Principal Investigator: Robert A. Lazzarini, Ph.D.

Other Investigators: Robert M. Winslow, M.D. and Kiyoi Nakata, Ph.D.

Cooperating Units: None

Man Years

Total:	3.0
Professional:	3.0
Other:	0.0

Project Description:

Objectives: The nucleic acid content of cells is subject to control by at least several regulatory mechanisms. The synthesis of certain species of messenger RNA is elicited by the presence or absence of specific metabolites in the cell milieu. The accumulation of ribosomal RNA, however, does not respond to the availability of specific nutrients but responds, rather, to the fitness of the medium and the growth rate of the cell. The cellular content of transfer RNA, on the other hand, responds neither to specific nutrients nor to the growth rate but is constant under most conditions of growth. The details of the mechanisms underlying these observations are at present obscure and are the subject of this project. We have concentrated on three aspects of these control mechanisms: 1) Rate of assembly of RNA chains. 2) Control of RNA synthesis in non-growing cells. 3) Coordinacy of RNA synthesis.

Methods Employed: Standard biochemical techniques are employed for the purification of RNA, DNA and enzymes. Analysis of complex mixtures of RNA are performed by chromatography on Sephadex G-100 or methylated albumin or polyacrylamide gel electrophoresis. Messenger RNA is estimated by molecular hybridization with homologous DNA. The rates of synthesis of individual RNA species are determined from the kinetics of incorporation of radioactive presursors into the species.

Major Findings: 1. Rate of assembly of RNA chains: The overall rate of RNA synthesis can be expressed as the product of the number of RNA chains under construction at any time and the average rate at which nucleotide residues are added to a single growing chain. Control of the rate of RNA synthesis could conceivably be mediated by mechanisms which alter the steady state number of RNA chains under construction, the rate at which these chains are elongated, or both. To decide between these alternatives we have developed a method for determining the steady state number of RNA chains under construction in cells during balanced growth. The second parameter affecting the rate of RNA synthesis, the rate of chain elongation, is obtained by dividing the total rate of RNA synthesis by the number of growing chains.

Using this analysis, we have demonstrated that the increase in the rate of RNA synthesis at faster growth rates is due solely to an increase in the number of chains under construction and that the rate of RNA chain elongation does not vary with growth rate. The nascent RNA chains in the cell are of two types, mRNA and stable (transfer and ribosomal) RNA. From our analysis we estimated that the distribution of chains between the two classes is 60% mRNA, 40% stable RNA. Furthermore, evidence has been obtained indicating that all RNA chains—messenger, ribosomal and transfer—are elongated at the same rate, 50 nucleotides per second at 37°C. The large number of nascent mRNA chains leads us to conclude that at least 20% of the total cellular mRNA is in the form of nascent chains.

2. Control of RNA synthesis in non-growing cells: When a stringent amino acid auxotroph is deprived of a required amino acid, growth ceases and the rate of uracil uptake into RNA is sharply restricted. However, relaxed derivatives of such organisms are able to assimilate uracil at near-normal rates, although growth is also inhibited. Various hypotheses have been proposed to explain the stringent response. However, any regulatory mechanism which decreases the rate of RNA synthesis must operate by reducing either the number of nascent RNA chains, or their rate of elongation, or both.

The rates of synthesis and chain elongation of RNA in stringent and relaxed strains of Escherichia coli have been measured in the presence and absence of a required amino acid. The following observations have been made:

A. Although the rate of uracil uptake by stringent strains is reduced 200-fold by amino acid starvation, the rate of RNA synthesis obtained using the specific activity of the intracellular UTP pool is reduced only 9-fold. The rate of RNA synthesis in relaxed strains is unaffected by amino acid deprivation.

B. The number of nascent RNA chains is approximately the same for both strains and is unchanged by amino acid starvation.

C. The rates of RNA chain elongation is reduced 8-fold during amino acid deprivation of a stringent strain. The rate for the relaxed strain is virtually unchanged by starvation.

These results taken together with those of the previous section indicate that RNA synthesis is regulated in at least two ways. During normal balanced growth RNA synthesis is regulated by the rate of chain initiation or by the steady state number of growing chains. During severe physiological stress such as amino acid deprivation the rate of RNA synthesis is curtailed by reducing the rate of chain elongation.

3. Coordinacy of RNA synthesis: The rate of RNA accumulation in most microorganisms is severely restricted when the organism is deprived of a required amino acid, pyrimidine, or purine base. A transient cessation of net RNA synthesis is also observed when cells are transferred from medium supporting rapid growth to one supporting slower growth (stepdown culture). We have undertaken a study to determine whether the synthesis of both messenger RNA and stable RNA are similarly affected under the restrictive conditions (coordinate control) or the synthesis of only stable RNA is curtailed (non-coordinate control).

Upon uracil deprivation of E. coli 15 TAU, RNA accumulation abruptly ceases. RNA synthesis, however, continues and is balanced by an equal degradation of RNA. The rate of total (message and stable) RNA synthesis, measured by adenosine incorporation, slowly declines during the course of uracil deprivation attaining a value of 16% of the control after one hour. Stable RNA synthesis, measured as methyl residue incorporation into RNA, also slowly declines during uracil starvation and attains a value of 12% after one hour. Consequently, the synthesis of message and stable RNA are controlled in an approximately coordinate manner during uracil deprivation.

The question of coordinacy of RNA control during amino acid deprivation and during stepdown culturing is currently being investigated.

Significance to Bio-medical Research and the Program of the Institute: Control of nucleic acid synthesis in bacterial systems represents examples of regulatory mechanisms which are at this juncture more amenable to experimental investigation than the more specialized mammalian systems. It is anticipated that information obtained from these simpler systems will help form a basis upon which to investigate the more complex regulatory mechanism operative in higher organisms and eventually to understand and control disease states of these systems.

Proposed Course of Project: 1. To investigate the parameters controlling the rate of RNA synthesis under conditions of restricted growth (amino acid deprivation, inhibition by drugs, etc.). 2. To study the co-ordinacy of the control of RNA synthesis. 3. To study the mechanism by which the number of growing RNA chains and rate of elongation are regulated.

Honors and Awards: None

Publications:

Winslow, R. M. and Lazzarini, R. A.: The rates of synthesis and chain elongation of ribonucleic acid in Escherichia coli. J. Biol. Chem. 244: 1128-1136, 1969.

Mantel, N., Winslow, R. M., and Lazzarini, R. A.: Isotopic labeling of messenger and stable ribonucleic acid and the specific activity of newly synthesized ribonucleic acid. J. Biol. Chem. 244: 1137, 1969.

Winslow, R. M. and Lazzarini, R. A.: Amino acid regulation of the rates of synthesis and chain elongation of ribonucleic acid in Escherichia coli. J. Biol. Chem. In press.

Lazzarini, R. A., Nakata, K., and Winslow, R. M.: Coordinate control of RNA synthesis during uracil deprivation. J. Biol. Chem. In press.

Serial No. NDS(I)-65 LMB/OC 1244
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Control Mechanisms and Differentiation

Previous serial number: SAME

Principal Investigator: Ernst Freese, Ph.D.

Other Investigators: Elisabeth Bautz Freese, Ph.D., Peter Fortnagel, Ph.D. (Guest Worker), Walther Klofat, Ph.D. (Guest Worker), Kin-ichi Sugae, Ph.D. (Visiting Associate)

Cooperating Units: Space Biology Branch, Goddard Space Flight Center, NASA (Dr. Grace Picciolo and Mr. Emmett Chappelle), and Laboratory of Microbiology, NIAID (Dr. Roger M. Cole)

Man Years

Total:	5.0
Professional:	2.7
Other:	2.3

Project Description

Objectives: Differentiation comes about by a multitude of individual biochemical reactions, each of which is subject to a specific control mechanism. Certain features of differentiation are common in micro- and macroorganisms and can best be studied in unicellular systems, such as the system of sporulation in Bacillus subtilis. Such common reactions are the repression of differentiation in growing cells, the commitment to differentiate once the process has started, the sequential control of developmental processes, and the control of DNA, RNA, protein and cell membrane synthesis. The pathways involved in differentiation can be analyzed in microorganisms by the isolation of developmental mutants and the analysis of their biochemical block.

Methods Employed: Sporulation mutants were obtained by treating bacterial spores with Cobalt^{60} and isolating colonies which were unable to produce the ordinary brown color. Sugar mutants were isolated by replica plating to plates containing different sugars as carbon source. ATP was determined by the luciferase reaction in a specially devised chamber and the carbohydrate enzymes were analyzed by coupling to NADH-dependent reactions or by other known techniques.

Major Findings: 1. ATP production as a tool to identify the biochemical lesions of mutants blocked in differentiation. Since sporulation mutants can grow on glucose medium, their biochemical deficiencies have to be identified by some means different from simple growth tests. We have first done this by measuring the incorporation of C^{14}-uracil into RNA but now have found that ATP determinations provide a much simpler assay system. The extraction of ATP is very rapid and the assay requires only two seconds. ATP is rapidly turning over in all cells because exposure to nitrogen reduces the ATP concentration by a factor of more than 20 within 20 seconds; this behavior is probably typical for strictly aerobic organisms, like B. subtilis, which cannot replenish significant amounts of ATP by fermentation. Whereas sporulating strains retain a high ATP concentration throughout the developmental period, most sporulation mutants are unable to do so. All sporulation mutants which we have so far isolated recover ATP synthesis when glucose or ribose are added to the medium; but some of them respond to all other carbon sources, whereas others respond only to certain carbon sources. Using the ATP test, mutants can therefore be classified according to the area on the biochemical map in which they are blocked. Mutant blocks in the citric acid cycle, in the Embden-Meyerhof pathway, and in some pathway of lipid metabolism have thus been identified. So far mutants blocked in the citric acid cycle have been examined in detail and some found unable to recover sporulation with the addition of different carbon sources either from the beginning or continuously. As long as ATP can be produced outside the citric acid cycle, sporulation is repressed, and when the outside sources have run out, not enough ATP can be made in the citric acid cycle to allow normal sporulation. The properties of other sporulation mutants are now under investigation with the hope to detect the mechanism by which the sporulation process is started. Particularly promising is one mutant which is able to sporulate in excess glucose which represses sporulation in the standard strain.

2. Control of NADH formation and sporulation in mutants requiring acetate. The experiments using citric acid cycle mutants had shown that the normal entry point of carbon sources during sporulation is via acetyl-CoA. In order to understand in more detail the significance of acetyl-CoA for growth and sporulation, mutants were isolated which required acetate for growth and which were blocked in the pyruvate dehydrogenase complex. In nutrient sporulation medium they grow to a low turbidity and do not sporulate subsequently. Acetate can restore both normal growth and sporulation. But some growth is also restored by addition of butanediol, citrate, glucose, glutamate, and certain other carbon sources but not with malate, oxaloacetate, or pyruvate. Several experiments have shown that the reason for this lack of growth is a deficiency in NADH which apparently sets in when the concentration of acetyl-CoA decreases below a critical value. The pyruvate dehydrogenase complex is therefore essential to maintain a sufficient NADH concentration during sporulation.

3. Antibiotic production by sporulating and non-sporulating cells. At the end of growth B. subtilis starts to produce an antibiotic activity against Staphyloccocus aureus. Sporulation mutants that are blocked very early in the

developmental process are unable to produce this antibiotic, in contrast to mutants blocked at a later stage. In turn, we have isolated several mutants unable to produce the antibiotic activity and found that they cannot sporulate. These findings suggest that the antibiotic activity may be required for sporulation. We have therefore purified this activity and measured its effect on B. subtilis itself. To our surprise we found that the antibiotic activity, which is produced during the developmental period, inhibits growth and causes lysis of B. subtilis when it is added during the vegetative period. This shows clearly that the antibiotic activity does act on B. subtilis itself; its mechanism of action is under investigation.

4. The importance of glucosamine for sporulation. Externally-added glucosamine represses sporulation of B. subtilis and yet mutants lacking the ability to produce glucosamine-6-phosphate are unable to sporulate. When these mutants are grown in the presence of glucosamine, this compound can still be used as a repressor of sporulation because a glucosamine-6-phosphate deaminase gives rise to fructose-6-phosphate and other Embden-Meyerhof compounds. Even when small concentrations of glucosamine are continuously fed to such cells during the developmental periods, only normal spores are formed which are unable to germinate. These results show that the concentration of glucosamine derivatives is delicately balanced in normal cells such that a very small concentration of glucosamine is available for sporulation but not enough to allow repression. The developmental consequences of a glucosamine mutant cannot be eliminated by the addition of small concentrations of glucosamine.

Significance to Bio-medical Research and the Program of the Institute: The problem of differentiation is one of the major unsolved problems in biology. It is likely that the mechanisms responsible for differentiation in microorganisms are essentially the same as those leading to the initial events of differentiation in higher organisms. Sporulation in bacilli is therefore merely a convenient system in which the principles of differentiation can be uncovered rapidly and can be proven rigorously by the use of biochemical differentiation mutants. The importance of this work to the institute is apparent, because most neurological diseases involve hereditary abnormalities of differentiation.

Proposed Course of Project: The next task will be to analyze the biochemical events which are necessary for the initiation of sporulation; this can be approached in several ways: Some sporulation mutants are blocked before the first stage of differentiation because they cannot produce the antibiotic activity. They are also deficient in some step of the lipid metabolism, which provides a handle for the identification of their biochemical block. The antibiotic activity itself can be purified and its effect on sporulation mutants can be tested. A minimal medium can be determined in which sporulation is possible and the significance of the compounds required spe-

cifically for differentiation can be determined. Particularly promising is a mutant capable of sporulating in the presence of excess glucose; glucose normally represses sporulation in the standard strain.

Honors and Awards: E. Freese, speaker at a symposium on sporulation in Urbana, Illinois.

Publications:

Schmitt, R.: Analysis of mutants deficient in α-galactosidase and thiomethylgalactoside permease II in Escherichia coli K-12. J. Bact. 96: 462-471, 1968.

Fortnagel, P. and Freese, E.: Inhibition of aconitase by chelation of transition metals causing inhibition of sporulation in Bacillus subtilis. J. Biol. Chem. 243: 5289-5295, 1968.

Schmitt, R. and Freese, E.: Curing of a sporulation mutant and antibiotic activity of Bacillus subtilis. J. Bact. 96: 1255-1265, 1968.

Freese, E., Fortnagel, P., Schmitt, R., Klofat, W., Chappelle, E., and Picciolo, G.: Biochemical properties of sporulation mutants. In Campbell, L. L. (Ed.): Spores IV, A Symposium held at Urbana, Ill. 3-6 October, 1968. Ann Arbor, Mich., American Society for Microbiology. In press.

Freese, E., Klofat, W., Picciolo, G., and Chappelle, E.: Production of adenosine triphosphate in normal cells and sporulation mutants of Bacillus subtilis. J. Biol. Chem. In press.

Freese, E. B., Cole, R., Klofat, W., and Freese, E.: Growth and enzyme defects of glucosamine mutants of Bacillus subtilis. J. Bact. In press.

Serial No. NIH (I)-68 LMB/OC 1552
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Integrative Control of Macromolecular Synthesis

Previous Serial Number: SAME

Principal Investigator: Michael Cashel, M.D., Ph.D.

Other Investigators: None

Cooperating Units: Laboratory of Nutrition and Endocrinology, NIAMD

Man Years:

Total:	2.3
Professional:	1.0
Other:	1.3

Project Description:

Objectives: Normal cells maintain a balanced composition of their macromolecular components during growth by integrative control of the formation of RNA, DNA, protein and structural elements. In general such regulation might be exerted by mechanisms which control: 1) the polymerizing enzyme(s), 2) the availability of low molecular weight precursors, and 3) the rate of breakdown of the macromolecular components. Two or more of these modes of regulation might operate in concert.

The dependence of RNA synthesis upon a complete array of amino acids is an exemplary regulatory phenomenon, since this process serves to integrate the formation of RNA with the synthesis of protein. Although the phenomenon is demonstrable in higher organisms, it is particularly amenable to study in Escherichia coli. The aim of this investigation is to understand the regulatory relationship between protein synthesis and RNA synthesis.

Methods employed: In addition to conventional microbiological, biochemical, and isotopic techniques, an improved method of thin-layer chromatography was developed for the resolution of nucleotides. The latter method permits quantitative resolution of the major acid-extractable ribonucleotides from crude extracts containing large amounts of $^{32}P_i$ and thus allows their direct analysis without intervening purification steps.

Major Findings: 1. Amino acid control of nucleoside triphosphate synthesis. Previous project work led to the suggestion that the direct regulation of RNA polymerase activity by amino acid availability (mechanism 1) was not of major significance. Instead, evidence implicated amino acid regulation of the synthesis of the ribonucleoside triphosphate substrates of RNA polymerase (mechanism 2), as follows. In the presence of CTP, ATP, and GTP, the incorporation of labaled UMP (but not of UTP) into RNA was strongly amino acid-dependent. Mutant cell strains (whose RNA accumulation is amino acid-independent) incorporation of UMP into RNA. It was demonstrated directly that the amino acid dependence of the conversion of UMP into UTP was similarly subject to amino acid control.

^{32}P activity measurements on the labeling of UTP, CTP, ATP, and GTP revealed that amino acid availability could regulate labeling of all four RNA polymerase substrates. More mild conditions of amino acid deprivation result in regulation of only GTP and ATP. The observed changes were considered not likely to be the consequence of RNA control by an independent mechanism, since inhibition of RNA accumulation by means other than amino acid starvation did not mimic the effects of amino acid starvation on the labeling of RNA polymerase substrates.

2. Implication of unusual nucleotides. Since control of RNA accumulation seems exerted at the level of availability of one or more precursors of RNA, it is necessary to establish how this occurs. A possible clue to this mechanism was provided by the observation of two unusual compounds (designated MS I and MS II), which appear heavily ^{32}P-labeled under conditions of amino acid starvation where labeling of all other detectable acid-extractable phosphorylated metabolites is reduced. Prior to extensive purification of these compounds it was necessary to determine whether amino acid regulation of the MS compounds was consistent with the notion that the compounds might participate in the amino acid control of RNA.

In addition to the pattern of more active labeling of MS compounds during amino acid starvation (which is correlated with the slowing of RNA synthesis), regulation of MS can be correlated with the effects of the following factors which influence the RNA control mechanism: 1) the effects of the allelic states of the RNA control gene, 2) the lack of specificity for the particular amino acid for which starvation is imposed, 3) the effects of interference with aminoacyl tRNA synthetase activity, 4) the effects of amino acid resupplementation of starved cells, and 5) the concentration-dependent effects of chloramphenicol addition to starved cells. In each case where the amino acid RNA control mechanism is likely to mediate the slowing of RNA accumulation, the amino acid MS control mechanism responds by producing MS I. Although most strains produce MS II under the same conditions as MS I, a class of strains with normal RNA control properties has been found which produces only MS I. Therefore, MS II can be dispensable in the RNA control process. Kinetic measurements made after amino acid starvation indicate that the appearance of the MS compounds is significantly faster than the slowing of uracil incorporation into RNA during a 20-second pulse. This observation

makes unlikely the possibility that MS compounds are merely a consequence of the slowing of RNA synthesis. Altogether, this approach indicates that the MS regulation mechanism and the RNA control mechanism are intimately related if not one and the same.

3. Purification and characterization of MS I. Because MS I seems invariably associated with the phenomenon of amino acid regulation of RNA accumulation, methods of purifying the compound from acid extracts of 300-liter amino acid-starved cultures were devised. Modifications of conventional procedures for nucleotide purification were necessary because of the unusual sensitivity of the compound to acid and alkali. Currently, about five μmoles of chromatographically pure material have been obtained, as well as radiochemical preparations labeled with ^{14}C or ^{32}P.

Preliminary analysis indicates that MS I is a guanosine derivative (chromatographic, biological, and spectral assays) containing four phosphate residues (chemical assay). Two of the phosphates in a pyrophosphate linkage are located at the 5' position of ribose (deduced from the products of hydrolytic attack by venom phosphodiesterase). The remaining two phosphates are also likely to be in pyrophosphate form at the 2' or 3' carbon of ribose (deduced from data concerning alkali degradation, insensitivity to periodation, and charge considerations). The tentative structural assignment for MS I is guanosine 5'-pyrophosphate, 2'- or 3'-pyrophosphate. Dipyrophosphoryl nucleotides have not hitherto been described in biological systems, nor have they been synthesized chemically.

Early attempts to characterize MS II, which is even more labile than MS I, suggest that it is structurally related to MS I, since one of the MS II degradation products is identical with MS I.

The availability of purified MS I preparations now makes possible a more critical appraisal of whether MS compounds cause the amino acid regulation of RNA accumulation. If the MS I compound is active in this respect, then its addition to defective mutants should cure their regulatory defect. Preliminary experiments indicating that this is indeed the case are qualified, because impure preparations of MS I were employed; such experiments are now being repeated.

Preliminary evidence also suggests that MS I is synthesized by chloramphenicol-inhibitable processes (presumably of protein synthesis) operating under a deficiency of charged aminoacyl-tRNA. If a protein synthetic site produces MS I *in vitro*, then this approach might further our understanding of the intricacies of protein synthesis as well as the integrative control processes relating the formation with the formation of RNA.

Significance to Bio-medical Research and the Program of the Institute: Amino acid control of RNA synthesis has been demonstrated in a variety of organisms, including human cells in culture. The phenomenon serves as a model

system for understanding how cells coordinately regulate the synthesis and function of their heterogeneous chemical components. In addition, the phenomenon of amino acid control of RNA has led to the establishment of relationships between RNA synthesis, protein synthesis, energy control systems, and transport of compounds across cell membranes; these areas of inquiry have recently proved themselves particularly relevant to neurochemistry.

Proposed Course of Project: To assess the mechanism of synthesis of MS I, its mode of action on phosphorylation processes and transport, its effect on RNA synthesis, and a more detailed structural determination of the compound.

Honors and Awards: None

Publications:

Cashel, M., Lazzarini, R. A., and Kalbacher, B.: An improved method for thin-layer chromatography of nucleotide mixtures containing ^{32}P labeled orthophosphate. J. Chromatog. 40: 103-109, 1969.

Cashel, M. and Gallant, J.: Two compounds implicated in the function of the RC gene of Escherichia coli. Nature 221: 838-841, 1969.

Cashel, M.: The control of RNA synthesis in Escherichia coli IV: relevance of unusual phosphorylated compounds from amino acid starved stringent strains. J. Biol. Chem. In press.

Serial No. NDS(I)-69 LMB/OC 1726
1. Lab. of Molecular Biology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Regulation of Dihydrofolate Reductase

Previous serial number: NONE

Principal Investigator: Robert Berberich, M.D.

Other Investigators: None

Cooperating Units: Mark Levinthal, Ph.D., Lab. of Molecular Biology, NIAMD

Man Years

Total:	1.4
Professional:	1.0
Other:	0.4

Project Description:

Objectives: Our current knowledge of the regulation of protein synthesis has come largely through the intensive study of a relatively few model systems in bacteria, bacteriophage, and, in the last few years, cultured mammalian cells. From the standpoint of regulation, three classes of proteins have been described: 1) inducible, in which the rate of synthesis of the protein increases in response to a specific inducer; 2) derepressible, in which the synthesis of the protein increases in response to the deprivation of a specific end product; and 3) constitutive, in which the protein is not under physiological control by metabolites, and is synthesized at a fixed rate under all growth conditions. It is postulated that different constitutive proteins are not synthesized at the same rate, but that a regulatory mechanism exists so that the rate of synthesis of each protein is adjusted to the requirement of the cell for its product. The regulation of dihydrofolate reductase is of interest because it appears to be an example of the third type of regulation, about which the least is known. Mutants with increased levels of the enzyme can be isolated in both bacteria and cultured mammalian cells by selecting strains that can grow in presence of the antileukemia drug, methotrexate, or similar drugs such as amethopterin and trimethoprim, all of which act by specifically inhibiting dihydrofolate reductase.

Methods Employed: The specific activity and catalytic properties of dihydrofolate reductase were measured in extracts of bacterial cells by a

standard spectrophotometric assay. Strains with increased levels of dihydrofolate reductase were isolated as trimethoprim resistant colonies after either no mutagenic treatment or treatment with ethyl-methane sulfonate (EMS) or 2-amino purine (2-AP). Genetic mapping experiments were performed in both Salmonella typhimurium and Escherichia coli using sexual conjugation and transduction.

Major Findings: 1. The constitutive nature of dihydrofolate reductase: Dihydrofolate reductase is a biosynthetic enzyme whose product, tetrahydrofolic acid, functions as a co-factor in wide variety of reactions and as a substrate in the synthesis of thymine. Unlike other biosynthetic enzymes, such as amino acid biosynthetic enzymes, it is not subject to repression or derepression. This has been established in a number of ways. For example, under conditions where enough trimethoprim was added to reduce the doubling time of the culture from one hour to three or four hours, no derepression of dihydrofolate reductase was observed. Since trimethoprim potently and specifically inhibits dihydrofolate reductase, it was concluded that the level of enzyme in the cell cannot be changed by interfering with its function.

2. Isolation of mutants with increased dihydrofolate reductase: Twenty-four independent mutants of S. typhimurium and three of E. coli were isolated. The maximum increase in the level of dihydrofolate reductase obtained in a single-step was 25-fold above the parent organism after using either spontaneous or EMS mutagenesis. Higher levels were obtained, however, by subjecting the cells to several rounds of selection on increasing levels of trimethoprim. One spontaneous mutant strain with a 600-fold increase in enzyme level was obtained after four rounds of selection.

3. Alteration of both the catalytic site and the amount of dihydrofolate reductase in three mutants: Three independent trimethoprim resistant mutants with elevated dihydrofolate reductase were found which also had a variety of alterations in the catalytic site. The first had a 20-fold increase in specific activity of dihydrofolate reductase and the pH optimum was shifted from 7.5 to 6.5. The second had a 2-fold increase in specific activity and a similar shift in pH optimum but had lost the ability of the parent organism and the first mutant to bind trimethoprim stoichiometrically. The third had a 4-fold increase in specific activity, a shifted pH optimum, an inability to bind trimethoprim stoichiometrically, and also an elevation in K_m for dihydrofolic acid.

The turnover number for dihydrofolic acid of the first mutant described above and the parent organism were determined by taking advantage of the ability of these proteins to bind trimethoprim stoichiometrically. The turnover number for both the parent and the mutant were the same, and it was concluded that the increased specific activity of the mutant is

a result of an increase in the rate of synthesis of the enzyme rather than an increase in the turnover number of the protein. This distinction could not be made in the case of the other two mutants since they had lost the capacity to bind trimethoprim stoichiometrically.

The simultaneous alteration of the structure of a protein and its rate of synthesis can be explained in either of two ways: 1) both effects are due to a single mutation; this is compatible with a role for a translational mechanism in the regulation of this enzyme; or 2) both effects are due to two mutations, which happened simultaneously in the same selective step, one affecting the structure of the protein and the other leading to an increase in the rate of its synthesis. This is compatible with a role for any of several previously described regulatory mechanisms in the regulation of dihydrofolate reductase.

4. Genetic mapping of mutations affecting the structure and amount of dihydrofolate reductase: by means of transductional analysis, the trimethoprim resistance marker (tmp A) of all twenty-four S. typhimurium strains was shown to lie between pyr A and ara at approximately four minutes on the genetic map constructed by Sanderson, so that the map order is pyr A tmp A ara leu. Tmp A is about 5% cotransducible with ara, 5% with pyr A, and 3% with leu.

In order to test whether the tmp A site was entirely responsible for all the trimethoprim resistance observed in each of the twenty-four S. typhimurium strains ten trimethoprim resistant cotransductants selected for leucine were tested by a streak test for their level of trimethoprim resistance. The transductants of nineteen strains had donor-type trimethoprim resistance but those of the other five strains had less than donor-type resistance. This indicates that one or more genetic sites in addition to tmp A can affect the level of dihydrofolate reductase. Assays of dihydrofolate reductase performed on extracts of both classes of transductants demonstrated that the trimethoprim resistance tests reflected actual differences in enzyme level. Donor-type transductants had enzyme levels equal to donor strains, and less than donor-type transductants had enzyme levels less than the donor strains. From this data it was determined that the second site(s) produced a relatively small effect on the enzyme level — an average of 4-fold among the five cases examined, which may explain why it only occured together with a mutation at tmp A. Moreover, all five cases which contained this class of mutation grew slowly in minimal glucose medium as compared with only two slow growers among the nineteen strains with mutations only at tmp A. The map position of the second site(s) is currently under investigation.

In order to determine the map position of the structure of dihydrofolate reductase, tmp A transductants derived from each of the three strains with structure and amount alterations were assayed for both properties and found to be identical in both respects to the donor strains. This indicates

that the structural gene for dihydrofolate reductase is cotransducible with the site affecting the amount of enzyme synthesized.

5. Summary and interpretation of data: Dihydrofolate reductase is a constitutive enzyme synthesized at a fixed rate in the normal cell and not responsive to the physiological manipulations which trigger changes in enzyme level in derepressible systems. Nevertheless, two classes of regulatory mutations affecting the level of dihydrofolate reductase can be obtained: 1) mutations at a major site, producing relatively large fold increases in enzyme level, and either identical to or tightly linked to the structural gene; and 2) mutations at a minor site(s), producing relatively low fold effects on enzyme level, and in addition, slow growth of the organism.

This pattern can best be explained in either of two ways: (1) Translational control: by this hypothesis the major site would be identical to the structural gene for dihydrofolate reductase and mutations at the minor site(s) might be altering a transfer RNA which increases either the rate of initiation or translation of the dihydrofolate reductase messenger RNA. (2) Transcriptional control: by this hypothesis, mutations at the major site are changes in a site (promotor) which binds RNA polymerase, thus affecting the rate of initiation of transcription of the dihydrofolate reductase gene. In this context, the second site(s) might affect the RNA polymerase itself, or some modifying factor, so that its binding to the dihydrofolate reductase promotor is increased.

Significance to Bio-medical Research and the Program of the Institute: The control mechanisms of protein synthesis in simple systems are important in themselves and have formed the basis for current investigations on similar problems in mammalian systems. The control mechanisms of individual enzymes and small groups of enzymes in bacterial systems have been useful models for the investigation of the far more complex regulation of protein synthesis that takes place in development and in response to hormones. In addition, the information obtained on trimethoprim resistant mutants of bacteria may be useful in understanding the resistance which develops in leukemia patients and others being treated with methotrexate.

Proposed Course of Project: 1) Complete cis-trans analysis of tmp A, already in progress; 2) map position of unmapped regulatory site(s).

Honors and Awards: None

Publications: None

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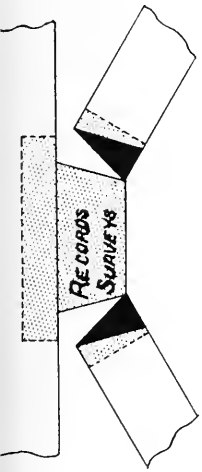

TABBED SEPARATOR SHEET
Form HEW-69
(3-56)

Summary Report
July 1, 1968 through June 30, 1969
Laboratory of Perinatal Physiology
National Institute of Neurological Diseases and Stroke

Ronald E. Myers, Chief

The Laboratory of Perinatal Physiology has studied perinatal brain damage utilizing experimental animal models. It also has investigated the broader issue of deficiency of energy availability to the brain and its implications for brain pathology utilizing both fetal and adult monkeys.

Experimental hydranencephaly has been produced in the rhesus monkey. Monkey fetuses were removed from the uterus at different gestational ages, the carotid arteries and jugular veins ligated and the fetuses restored to the uterus. At later delivery, the brains were examined for pathological changes where a variety of outcomes obtained.

The earlier in gestation ligation was carried out the more likely the production of severe morphological changes in the brain.

Classical hydranencephaly occurred in this study. More commonly, however, there was infarction in the supply areas of the anterior and middle cerebral arteries. With later ligation cerebral dysgenesis was seen characterized by abnormal fissurational patterning and gross distortion of the cerebrum. These morphological changes resembled those exhibited by the brains of children with a severe type of retardation.

Fetal survival occurred in 54 percent of cases. This represents a high rate of fetal survival following fetal surgery. More recently, however, the techniques of fetal surgery in our laboratory have improved to the extent that fetal survival obtains in 90 percent of cases.

Episodes of total asphyxia result in patterns of pathology restricted to the brain stem. Recent efforts concern ways of preventing asphyxial brain damage. Barbiturate anesthesia significantly protects the nervous system when it is insulted by episodes of total asphyxia. The tolerance to asphyxia is extended 30% by the barbiturate depression of CNS metabolism. Similar studies have established the importance of hypothermia and of correction of asphyxial acidosis in protecting the rhesus monkey infant against asphyxia.

Umbilical blood flow may be obstructed for measured lengths of time in fetuses of different gestational ages. A variety of pathological changes have resulted, the type depending upon the duration of cord compression and fetal gestational age. The earlier in pregnancy asphyxiation occurs the more tolerant is the animal to the insult (3-fold differences in tolerance seen). When minimal pathology results at different gestational ages, the patterns produced compare closely with that found with an episode of total asphyxia at term; e.g., bilateral symmetrical brain stem damage. However, the younger the fetus the more relatively preserved are the more rostral CNS structures.

When more severe episodes of asphyxia are delivered to developing fetuses, increasingly severe damage to the brain results until the entire CNS may be destroyed, resulting in a cystic transformation of the brain. In some loci there is connective tissue replacement of the necrotic tissue. Other zones may exhibit only persistence of coagulation necrosis. Still other zones may show a complete destruction of their neurons but with preservation of the glial stroma.

The effects of prolonged partial asphyxia on the fetal and newborn nervous systems have been characterized. Swelling of the brain occurs with in utero asphyctic compromise. When the insult is sufficiently severe, hemispheral necrosis may result. The necrosis is sometimes hemorrhagic, sometimes non-hemorrhagic. Usually, when cerebral necrosis occurs, the assault has been severe enough that survival beyond the first hours after delivery is jeopardized. However, in those animals in which the brain destructive process and the asphyctic compromise is not so severe as to jeopardize life, survival beyond 3 months is associated with a pattern of brain pathology resembling that occurring in humans suffering perinatal injury; e.g., atrophic cortical sclerosis, white matter sclerosis, and status marmoratus of the basal ganglia.

Thus, studies suggest the human exhibiting perinatal brain damage has sustained an episode of prolonged partial asphyxia. Such an insult may occur in utero under a variety of circumstances or it may occur during the birth process itself.

The threshhold levels for the occurrence of brain swelling and brain damage have been determined. Levels of pH ranging between 6.9 to 7 lasting up to 3 hours are required to produce brain damage. Such an animal will exhibit a pCO_2 of 90 or above, a pO_2 of 15 or less, a base deficit of -18 or greater and will maintain blood pressures of a mean arterial value of

40 mm. Hg. or less and an oxygen saturation of 15 percent or less. Animals with values better than these will not exhibit evidence for brain damage.

Saling in the early 1960's developed a technique of evaluation of fetal status involving sampling of fetal scalp blood during labor. This technique allows the precise determination of the acid-base and respiratory gas status of the fetus during birth. However, the validity of this technique depends on the accuracy with which the blood from this site reflects the status of the fetus.

A study has been completed revealing an astonishing closeness of fit of blood values as obtained from the fetal scalp and the aorta and superior vena cava. Thus, the fetal scalp sample appears to accurately reflect the acid-base status of the fetus.

The neuropathology of stillbirth in the monkey has been examined further. The most frequently occurring patterns of pathology include swelling of the brain associated with a hemorrhagic retinopathy; swelling of the brain associated with a gross disruptive laminar necrosis in the cerebral cortex and/or cerebellum; swelling of the brain associated with cerebral softening or necrosis; and perivenular white matter hemorrhage in prefrontal and posterior parietal regions.

Models of hypoxic brain damage in the adult rhesus monkey have been developed. In early experiences with this model, brain swelling was frequent. However, hypoxia itself seriously affects the status of the heart. As a result, hypotension tends to develop resulting in varying degrees of cerebral malperfusion. The impaired perfusion of the brain causes a distortion in the brain physiological and pathological changes produced by the hypoxia itself. With increasing ability to maintain the blood pressure levels above a critical level, severe degrees and prolonged lengths of hypoxia have been found to be tolerated by the rhesus monkey (with partial pressures of oxygen down to 18 to 20 mm. Hg. lasting for as long as a half hour) without producing brain swelling or brain damage. These findings raise serious doubts regarding many present concepts regarding hypoxia and CNS integrity.

In the earlier studies, extensive necrotic lesions affecting the white matter were found. Again, as the hypoxic model has been perfected, this pattern of pathology has completely

disappeared. It is inferred that the white matter necrosis results from a combination of severe tissue hypoxia and some degree of cerebral tissue malperfusion. With the perfected model, where mean perfusion pressures are maintained above 60 mm. Hg., lesions of the brain, when they occur, are limited to the globus pallidus.

Studies have been carried out attempting to characterize the physiological and biochemical changes associated with hypoxia in the adult. In hypoxia produced by respiring animals with 2 1/2 to 3 1/2 percent oxygen mixtures, the EEG becomes iso-electric after 3 to 8 seconds. The arterial pO_2 in such animals is 18 while the jugular venous pO_2 is 12. Both the arterial blood and the cerebrospinal fluid develop a severe acidosis of a metabolic type. Along with the developing acidosis, the cerebrospinal fluid exhibits a significant decrease in bicarbonate level.

Importantly, administration of 100 percent oxygen at termination of the insult results in a dramatic increase in jugular venous levels of oxygen. Jugular oxygen partial pressures may range from 275 to 310 mm. Hg. (normal levels with 100% oxygenation approximate 35 to 40 mm. Hg.). The duration of the jugular venous hyperoxygenation following such an insult directly relates to the duration of the hypoxic insult itself providing the arterial blood pressure has been adequately maintained. When arterial hypotension occurs, the degree and duration of jugular hyperoxygenation is considerably increased. Thus, hypotension per se represents a significant added insult to that of the hypoxia. The increased partial pressures of oxygen in the jugular venous blood in the animals following the period of insult when respired with 100 percent oxygen indicates the presence of a combination of a diminished CNS energy metabolism resulting in a decreased oxygen utilization and of an increased cerebral blood flow resulting from a disturbance of autoregulation produced by the hypoxia.

Cerebral impedance in relation to hypoxia increases by 20 to 30 percent with an onset latency of 2 to 4 minutes. The CNS impedance returns to baseline levels rapidly after the administration of 100 percent oxygen, providing significant arterial hypotension did not occur.

The physiological changes associated with total asphyxia in the adult animal have been studied. Primary attention is focused upon the acid-base and respiratory gas alteration in the blood versus the cerebrospinal fluid. Three procedures were

carried out: 1) total asphyxia produced by tracheal clamping; 2) washout with nitrogen maintaining a normocapnic state; and 3) nitrogen washout with hyperventilation producing a hypocapnic state. In all three circumstances a striking increase occurred in the hydrogen ion concentration in both the blood and cerebrospinal fluid. This increase was considerably less in the hypocapnic state than in the other two models. With hyperventilation, the animals exhibited a circulatory collapse and death while blood pH's were still strikingly alkaline (7.6). However, even in this circumstance the CSF pH was 7.1. In these animals a circulatory collapse occurred within the first 5 minutes. The animal's circulation ceased between the 5th and the 10th minute at which time the measurements of blood chemistry no longer reflected tissue states.

Adult monkeys tolerate a complete arrest of the systemic circulation for up to 20 minutes with near complete clinical recovery. Circulatory stasis is produced by clamps placed on the superior and inferior vena cavae and on the ascending aorta. However, brain lesions first appear after a circulatory arrest of 14 minutes. The pathological changes of uncomplicated circulatory arrest consist of lesions in the brain stem, the inferior colliculus, the cerebellar vermis, ventral thalamic nuclei, trigeminal sensory nuclei, etc., being most vulnerable. Thus, the pathology of uncomplicated circulatory arrest in the adult monkey closely compares to that produced by total asphyxia in the newborn.

The circulatory arrest model also is fraught with difficulties. Frequently, following arrest, the animals exhibit severe hypotension which may endure for a considerable time. In these cases the neuropathological outcome is altered consisting of diffuse spotty lesions of cortex and often severe basal ganglia lesions. Such lesions do not occur after uncomplicated circulatory arrest.

Hypoglycemia induced by insulin represents a special circumstance in which energy sources are denied to the nervous system by virtue of substrate withdrawal. Glucose serves as the primary, if not exclusive energy source to nervous tissue. The hypoglycemic model of CNS hypoergia is most satisfactory because of the lack of the significant circulatory changes produced by other forms of energy deprivation. Also, unlike hypoxia or anoxia, hypoglycemia is not associated with a metabolic acidosis. Thus, through careful control and surveillance of the preparation, it is possible to deprive the CNS of energy availability without producing the usual striking changes in circulatory or in acid-base or respiratory gas status.

Experimental hypoglycemia has resulted in two distinct patterns of brain pathology. One exhibits severe destructive well-demarcated lesions involving primarily the basal ganglia. The second consists of diffuse nerve cell loss associated with astrocytic gliosis and diffuse cytological changes of preserved neurons.

Hypoglycemia results in a severe depletion of CNS glycogen over several hours. With restoration of blood glucose, the brain glycogen is restored over several hours but continues climbing to reach peak levels, 2-3X normal, at 12 hours and remains elevated for several days.

During hypoglycemia CNS polysomes undergo disaggregation leaving only monosomes. A disaggregated state persists through the first hours following the restoration of glucose. Polysomal reaggregation is not complete even at 12 and 24 hours post-insult. Associated with polysomal disaggregation are severe states of CNS depression and malfunction.

An earlier histochemical study demonstrated a remarkable increase in glycogen content of astrocytes both of gray and white matter at 10 hours after an episode of acute total asphyxia in the term fetus. In more recent studies the glycogen content of brain and other organs was determined using biochemical techniques.

The investigation was extended to 1) monkey newborns subjected to acute total asphyxia; 2) juveniles subjected to episodes of circulatory arrest; and 3) juveniles undergoing episodes of severe hypoglycemia. In all instances the episodes of CNS energy deprivation resulted in a rapid breakdown of glycogen content to 30% in cerebral tissues. Following the insults, a rapid recovery to normal levels occurred by the third hour in all instances. The content continued to increase to maximum values at 12 hours (to a two-fold increase over normal). In the asphyctic models the glycogen returned to normal over the next 24 to 48 hours. However, following hypoglycemia the content remained elevated for prolonged periods.

Deep barbiturate anesthesia results in a cerebral hypometabolism. Current studies reveal a striking increase in glycogen (3X normal) during deep barbiturate anesthesia suggesting a possible relationship between cerebral hypometabolism and glycogen increase at 12-24 hours. This finding offers a possible explanation for abnormal glycogen increase following CNS energy deprivation.

The ATP metabolism of the hypoxic cerebrum has been examined. Cortical ATP levels exhibit a 10-fold decrease soon after the onset of 3.5 percent oxygen administration. Thus, energy availability to nervous tissue is rapidly impaired in severe hypoxia. The time course of restitution of high energy phosphate following energy deprivation states is under examination.

A considerable increase occurs in the extracellular spaces of the brain following fetal asphyxial compromise. Such changes occur even in the absence of gross evidence for brain swelling. A net shift of fluid occurs from the intra- to the extracellular compartments. Striking ultrastructural changes also occur in the mitochondria which are grossly swollen and frequently exhibit rupture of the cristae. The neuronal ribosomal rosettes tend toward a breakdown and disaggregation.

In the area of developmental neurology studies have continued on placental insufficiency in sheep. The most significant alteration following experimental placental insufficiency is a decrease in liver weight, the decrease being proportional to the decrease in placental mass (or placental non-collagen protein). Less significant was the reduction in total body weight. No evidence for brain weight reduction could be noted. The tissues of these sheep are being extensively analyzed biochemically in collaboration with a group at Johns Hopkins. Organ DNA, RNA, trace metals, enzyme activities, glycogen contents, insulin levels in blood, etc., are being carried out. Thus far, findings include a decrease in the brain cell populations despite the absence of changes in brain weight. The RNA content of cerebellum is decreased. Myocardial cell hypertrophy occurs without an increase in myocardial mass or comparable changes in skeletal muscle. Similar studies are being carried out in the rhesus monkey. Clear-cut cases of fetal dysmaturity or fetal growth retardation have resulted from ligation of umbilical vessels as they pass from the umbilical cord to the placenta.

In the area of physiological psychology studies near completion of an analysis of the prefrontal and the anterior temporal neocortex in their relation to perceptual and mnemonic functions, on the one hand, and to social and emotional behavior on the other. Effects of lesions have been tested on a variety of problem box performances, including delayed response, go-no-go visual color discrimination, simultaneous object color discrimination, pattern discrimination, pattern discrimination reversal, extinction tests, and position habit reversal. Curiously, lesions either of anterior temporal or of prefrontal cortex produced

deficits not seen with lesions of the thalamic dorsomedial nucleus. The configuration of test performance deficits observed with prefrontal cortex removals are quite dissimilar from those found after lesions of temporal neocortex. These differences occur despite the several lines of morphological evidence suggesting a close anatomical interrelationship between the orbitofrontal and prefrontal cortex on the one hand and the anterior temporal neocortex on the other.

The prefrontal cortex supports delayed response, delayed alternation, and go-no-go visual discrimination performances. The interocular transfer of these visually oriented tasks in chiasma-sectioned animals may be inferred to depend upon the corpus callosum. However, current studies have shown that delayed alternation performance is transferred between the eyes at a high level despite total section of the forebrain commissure. Delayed response performance is also transferred between the eyes with considerable savings. On the other hand, go-no-go visual discrimination performance transfer is totally dependent upon the integrity of the forebrain commissure.

The effects of section of the forebrain commissure at birth and in later life have been compared with regard to touch, motor skill, and visual learning transfer. No crucial differences in transfer effects were noted indicating the absence of age effects in relation to section of the forebrain commissures.

Studies of corpus callosum have implicated this structure in the transfer of information underlying motor skill learning. Rhesus monkeys allowed to observe visually the learning of specific motor skills with one hand may exhibit a successful transfer of this learning to the other hand despite the prior section of the commissure. Thus, visual information alone can serve as an alternative mechanism permitting transfer of motor skill learning between the hands. Such may even occur in the absence of visual stimulation on transfer testing. Thus, even visual memories of prior learning experiences achieved through the opposite side of the body may mediate the transfer of learned motor skills.

The transfer of learned motor skills between the hands depends upon a specific portion of the corpus callosum. Curiously, the anterior boundary of this functional sector is located approximately 10 mm. posterior to the anterior tip of the genu while the posterior boundary is approximately 8-10 mm. anterior to the posterior tip of the splenium. Thus, both precentral and parietal

lobe portions of the commissure may equally handle information underlying transfer of learned motor skills. Further, an equivalence of these two portions of the commissure has been found in relation to this function. Thus, both the parietal lobe and the precentral gyrus appear to play crucial roles in motor skill learning and in its information transmission between the hemisphere. Anterior commissure makes no contribution to this function.

A lesion of the hand area of the precentral gyrus produces no effects on contralateral distal extremity use or strength until the lesion is a total one. With total hand area lesions motor strength and motor use drop to and remain at zero.

Animals which have sustained section of the corpus callosum and anterior commissure two years earlier no longer exhibit evidence for degenerating fibers using silver stains. Such animals are again subjected to craniotomy and the thalamus removed completely on one side through the area of prior commissure section. Following serial section of the brain and staining with the Nauta technique, the total pattern of thalamocortical projections may be studied. Heavy projections are seen from the thalamus to the visual, the auditory, and the tactual sensory projection areas. However, surprisingly, other areas of cortex receive thalamic fibers in surprisingly small quantity. Widespread areas of cortex exist that are either entirely free of projection fibers from the thalamus or receive them only in sparse numbers. Such areas include the anterior one-third of the temporal lobe, the associational areas of the visual mechanism including areas 18, 19, and 20 and 21. The posterior parietal lobe is poorly receptive of thalamic projections while the precentral gyrus receives them in great numbers. Details of this pattern remain to be worked out.

Vocalization in the rhesus monkey is not under the control of volitional brain mechanisms. It has not been possible to condition rhesus monkey vocalizations despite a wide variety of approaches utilized. A direct comparison of the rates of acquisition of bar press behavior, of an irrelevant backward head twist behavior, and of vocalization as instrumental modes of responding in relation to specific visual stimuli has been made. Bar press behavior was picked up rapidly while the specific head twist was acquired with moderate difficulty. Vocalization did not develop as an instrumental mode of responding.

Some animals vocalize spontaneously in the behavioral test situation. Cortical areas deemed homological in location

to the speech areas of the human were destroyed bilaterally in such animals. These lesions failed to produce alterations in the spontaneous vocalization patterns.

However, lesions of the anterior temporal or the prefrontal cortex did produce striking effects on spontaneous vocalization.

Major cortex lesions produce only slight effects on the control of contralateral facial expression in the monkey. Thus, total removal of the parietal or occipital lobes has no demonstrable effect on contralateral face use. Surprisingly, removal of temporal lobe produced a 30 percent deficit, while total frontal lobe lesions have only a 20 to 40 percent effect. Lesions of prefrontal cortex produce the same effect as total frontal lobe removal. Total removal of precentral gyrus may produce only slight effects. Thus, the prefrontal and anterior temporal cortex are the cortical centers having to do with facial expression in the monkey. These monkey results contrast strikingly with those in the human where only lesions of precentral gyrus are known to result in major losses of use of the face on the opposite side.

From the results it begins to appear that the monkey (in contrast to the human) has little volitional control of their facial expressions or vocalizations. However, the prefrontal and the anterior temporal cortex, which are involved with emotional or instinctual behavior, also regulate and control both facial expressions and vocalization in the monkey (see later discussion of effects of lesions of these structures on social behavior).

A study describing the normal reproductive behavior of the caged male rhesus monkey has been completed. Studies on the neural mechanisms underlying male reproductive behavior in the rhesus male indicates that lesions of anterior temporal cortex and of amygdala do not affect reproductive behavior. However, when the uncus including the amygdala is removed, a striking increase in reproductive behavior results with a dramatic increase in numbers of mounts and inseminations performed per hour. Lesions of olfactory tracts or of prefrontal cortex again failed to produce essential alterations in reproductive behavior but a great increase occurred in stereotyped behavior and in general activity levels following prefrontal lesions.

The effects of cerebral lesions on the social behavior of free-ranging rhesus monkeys have been explored on a single

social group on Cayo Santiago. Animals were subjected to lesions of prefrontal or of anterior temporal cortex. These ablations in both instances resulted in a failure of the animals to return to their social group on release. Instead, the animals remained solitary during their 2 to 16 week survival on the island.

Exceptions to this result occurred among yearlings subjected to comparable lesions. These animals failed to exhibit the striking deficit in social behavior, returning to their social group on release. On careful examination, they appeared only minimally altered in their interactions with other animals. The older animals, in addition to a failure to return to their social group, exhibited other changes in behavior including increased tameness on human approach, entry into areas usually avoided, and diminished aggressive displays. Animals with prefrontal removal exhibited hyperactivity and, generally speaking, survived a shorter period of time than those with anterior temporal lesions.

On Cayo Santiago the internal organization of Group A is under detailed examination. This group consists of 4 subgroups -- a central subgroup, 2 all-male subgroups, and a smaller mixed subgroup. One all-male subgroup is peripheral to the central group of Group A, traveling mostly in relation to that group. The second all-male subgroup, on the other hand, spends much time away from Group A proper but occasionally feeds with this group and participates with it in mutual self-defense. The smaller mixed subgroup acts in many ways as a separate social group usually traveling and feeding independently of Group A. However, in some respects this group continues to be a part of Group A. Occasional instances of mutual grooming of individual members of the two groups occur. Instances also occur of mutual defense against third groups. Instances of breeding between animals of these two groups have also occurred.

This smaller mixed subgroup consists of animals in the act of splitting off from the main body of Group A. However, no instances have occurred in which close relatives have been split between the two groups. Rather, splitting occurs only among distant relatives; the closest relationship split is that of cousins.

The social order of a second group has been characterized. Group C consists of 26 distinct familial or genealogical subgroupings. These subgroups are not entirely separate but express degrees of interconnectedness. Fifteen families exhibit a higher degree of interrelatedness among themselves as measured by interfamilial grooming than do the remaining 11 families. Within the

15 main families none remained isolated from the rest. Rather, any arbitrarily chosen subgroup is connected at several points to other subgroups. The remaining 11 families exhibit selective bonds throughout the network and with one another but these bonds appeared of weaker strength. These 11 families failed among themselves to form a distinct subgroup. Thus, group C maintained its cohesiveness through an intricate interlocking of interfamilial associational ties in which some families are linked more closely than other families. No central grouping existed which was predominant and which regulated the movement or activity of the band as such.

Detailed social studies of Group F have continued. These studies have documented the social interactions and behavioral patterns expressed by all members of this relatively small social group. Particular emphasis has been placed in the last year on the estrous cycles and estrous behavior of the females. For a detailed discussion of these studies, see project number NDS(I) 68-1558.

Daytime sleep and rest patterns have been examined in Group E. Sleep was defined as bodily immobility with closed eyes. The animals slept 6% of the time during the day. The average sleep interval was 25 seconds. Females slept more than males but failed to sleep in the early afternoon at a time when the males slept the most. Rapid eye movement sleep occurred rarely. Animal age was not correlated with sleep obtained. Animals tend to rest clustering with other family members. Animals orphaned during the second six months of life rest more often with adult males than with known relatives.

In La Parguera investigations continue on both free-ranging and enclosure-held monkeys. The maternal-infant dyad has been examined. Time spent by the infant at the nipple varies from 100 percent shortly after birth to a situation approaching zero by the end of the first year. Time spent in ventral-ventral contact diminishes sharply from 100 to 50 percent over the first 9 weeks. Thereafter, the rate of decrease in time at the breast diminishes more slowly. At the end of the first year of life, when the mother delivers her next infant, the yearling spends almost no time at the nipple. The newborn thus has full access to the nipple with no abrupt transition for the yearling.

The diminution in ventral-ventral contact between mother and infant occurs with a minimum of punishment of the infant by the mother. Thus, maturation of neural circuits with the development of interest and curiosity directed toward other objects in the environment appears to play the major role in the developing disassociation of the infant from the mother.

In play activity, sex differences developed and became striking during the second 6 months of life when infants played more with infants of their own than of the opposite sex.

Dominant adult males have been removed from their social groups for lengths of time varying from 2 days to 10 weeks. These removals resulted in no changes in the group hierarchy or in the patterns of movement. Removal of dominant males produced only subtle effects on group behavior in general. Second-ranking male frequently moved into the center of the group. With release of the originally dominant animal, aggression, grooming, mounting, following, and vocalizing greatly increased. Females were more successful in rejoining their groups than males, probably because young adult males frequently spontaneously switch groups whereas females almost never do. Low-ranking males and females were more likely to assume their former positions than were dominant animals. Thus, sex, rank, and time out of group are important variables in defining the consequences of removal and re-introduction of animals into free-ranging rhesus monkey groups.

Interactions between two social groups maintained in two separate experimental enclosures have been studied. Most interactions between the two were antagonistic. However, infrequent instances of play, presenting, following, and social mounting were observed. Grooming and copulation were absent between individuals of the two groups. A distinct pattern of dominance was established between the two groups.

Peripheral, immature males were the most frequent participants in intergroup interactions. By contrast, dominant males appeared detached and rarely participated in interactions. When peripheral males were removed from the dominant of the two groups, dominance reversal occurred. Dominance relationships between social groups appears to depend not on dominant males but on the peripheral subadult males.

In comparative neurology the development of neuro-histological methods has been one of the most active research areas, culminating in international conference on "Current Methods in Neuroanatomy." In this symposium new methods developed for the demonstration of degenerating axons and axon terminals were described. These methods are modifications of Nauta's original technique for the selective silver impregnation of degenerating plasm.

A project dealing with the distribution of ascending spinal fibers in a variety of vertebrate species has been

completed. Among the findings are (1) the demonstration of a direct spinal-cerebellar system in the dorsal funiculi in all non-mammalian vertebrates examined; (2) a connection between the dorsal column nuclei and the dorsal accessory olive in the cat and monkey; (3) the demonstration of a spino-thalamic connection in several reptilian species.

The comparative studies of the telencephalon have been limited to olfactory tract projections in the shark and interhemispheric connections in the lizard. Although it has been believed that the olfactory tract in the shark contributes fibers to the entire telencephalon, both ipsi- and contralateraly, it was found that its fibers reach only a relatively small, well-confined region on the ipsilateral side. The pallial commissural fibers in the lizard were found to distribute only to the medial, dorsomedial, and dorsal cortex whereas the more lateral cortical regions in these species apparently do not receive a commissural input. This is of some importance in relation to the evolution of neocortical structures since the dorsolateral cortex has been thought to be a primordial neocortex. The absence of a commissural input to this region suggests either that this is indeed a primary neocortical structure, but without a commissural component, or that this cortex is not related to neocortex as we know it in mammals.

The retinal projections in several fish have been described for the first time using the Nauta method. The findings suggest a common pattern of organization in non-mammalian vertebrates with a few small apparent variations in some species. It had been thought that the shark retina projects only to the thalamus and optic tectum; however, studies in this Laboratory indicate that there are projections to the hypothalamus, ectomammillary nucleus, and the pretectal nucleus as well. Similar findings were noted in teleost, amphibian, and reptilian species.

The studies on the mesencephalon are primarily related to the connections of the optic tectum which have been described for the first time in detail in the frog and the lizard.

The neurometric studies have dealt primarily with the evaluation of old methods and the development of new ones. Of particular concern during the last year has been the comparison of sampling methods for the counting of particles in histological preparations. Both the problem of the selection of sections and the areas with a given section have been dealt with. As might be expected, it was found that systematic sampling is more efficient in highly organized structures than

random sampling techniques. In situations where the population is randomly distributed, a random sampling technique is preferable.

Serial No. NDS(I) 65-LPP 1259
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Neuropathological effects of umbilical cord compression.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	.8
Professional:	.3
Other:	.5

Project Description:

Objectives: To determine the pattern of clinical and neuropathological change produced by umbilical cord compression. To determine the length of cord compression required for production of neuropathological change at different gestational ages.

Methods Employed: Hysterotomy with sterile delivery of fetus; fetal electrocardiography; catheterization of fetal vessels with recording of blood pressure; blood gas and acid base studies of fetal blood; compression of umbilical cord; return of fetus to uterus; fetal survival in utero; c-section on 158th day; assessment of clinical status of infant; survival into juvenile aged; and finally, gross and neuropathological study of CNS.

Major Findings: Depending upon length of cord compression and gestational age a variety of neuropathological changes are seen. The most severe is advanced cystic degeneration of the nervous system with destruction of the neural parenchyma of brain and spinal cord. Surviving neurons are seen only in dorsal root ganglia, myenteric plexus of the gut, and bipolar cells of the retina. Such brains exhibit complete necrosis with connective tissue replacement of the cortex and the white

matter of the hemispheres, coagulatory necrosis with small vessel thrombosis and without connective tissue replacement in thalamus and basal ganglia, and with destruction of the entire neural parenchyma without necrosis and with preservation of the astrocytic element in brainstem and spinal cord.

Such animals do not survive beyond birth. A lesser damage includes a clinical state compatible with survival through the newborn period but with evidence clinically for severe CNS involvement including severe changes in postural tone and in motor use. Neuropathologically, such animals exhibit severe destruction of neurons symmetrically throughout large portions of the tegmentum of the brainstem, destruction of entire complexes such as the superior olivary nuclei, the trigeminal sensory nuclei, vestibular nuclei, and inferior colliculi. These changes are associated with capillary proliferation, macrophagic response and astrocytosis. Still lesser degrees of damage produce a clinical status resembling spastic quadriparesis with a neuropathological picture associated with destructive changes similar to that just described but of lesser extent. Frequently such animals, despite severe destructive changes in brainstem, are found to lack pathological changes in the cerebrum itself. Finally, umbilical cord compression for as long as fifty minutes at earlier gestational ages and as long as twenty minutes at later gestational ages have resulted in no clinical abnormalities and no pathological changes in the brain. The tolerance to asphyxia, as determined by the length of asphyxia required for production of brain damage, has been plotted for fetuses of gestational ages from eighty days through one hundred and sixty days. The shape of the curve derived is logarithmic with fetuses of younger gestational ages exhibiting remarkably tolerant to asphyxia.

Significance: The present studies have clearly established the validity of using the fetal and newborn monkey as an experimental model for the study of perinatal brain damage in the human. To date each of the classical and better known patterns of neuropathological change found in the human in relation to asphyxial brain damage has been duplicated in monkey model. The study of the pathogenesis of these various patterns of brain pathology in the experimental animal should aid in the better understanding of the pathogenesis of cerebral palsy, mental retardation, and brain insult in general in the human.

Proposed Course of Project: More cases will be developed of cord compression in the experimental monkey in order to better establish the spectrum of pathological change produced

under these circumstances. These studies also enable the development of information with regards to tolerance to asphyxia and the length of asphyxia required to produce the varying degrees of brain damage.

Publications: None.

Serial No. NDS(I) 65-LPP 1261
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The superior colliculus in the goat.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: 0
Professional: 0
Other: 0

Project Terminated.

Publications: None

Serial No. NDS(I) 65-LPP 1262
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Reproductive behavior of caged rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigators: Mr. Luis R. del Río

Cooperating Units: None

Man Years:
Total: 2.3
Professional: 1.5
Other: .8

Project is completed.

Publications: None

Serial No. NDS(I) 66-LPP 1385
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 - June 30, 1969

Project Title: Pathology of the stillborn.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	.3
Other:	.9

Project Description:

Objective: Pathology of the stillborn monkey.

Methods Employed: All stillborn monkeys are subjected to postmortem examination to determine state of preservation of tissues and to describe the pathology of CNS.

Major Findings: The stillborn may be divided into fetuses with advanced tissue maceration with peeling of epidermis, liquefaction or softening of internal organs, and loss of normal tissue coloration and fetuses with preservation of intact gross morphology of internal organs. Intermediate circumstances are found, the most common being brain liquefaction with preservation of intact gross morphology of somatic organs. Several patterns of neuropathological change have been seen. Perhaps most common has been the occurrence of perivenular hemorrhages in the white matter of the hemispheres with predominance in the white matter of prefrontal lobe, the white matter of the posterior parietal lobe and extending into occipital lobe. White matter necrosis with liquefaction may occur. This pattern conforms with that seen in humans exhibiting periventricular leukomalacia. Laminar necrosis also is often seen which exhibits several types: 1. Universal gross disruptive laminar necrosis with greater

predominance in the depths of sulci. 2. Laminar necrosis within the neuronal laminae of the hippocampi. 3. Laminar necrosis occurring in cerebellum alone. The third is most common. Laminal necrosis is associated with gross brain swelling and hemorrhagic retinopathy. In addition to these types of pathology, there is a third, observed frequently in the stillborn fetuses, namely, severe brain swelling without evidence for the other pathology changes.

The state of preservation of CNS and somatic tissues bears little relation to length of postmortem survival in utero. Rather, the state of preservation of tissues and the degree of postmortem autolysis expresses appears more likely related to the rapidity of fetal death in utero (see studies on chemistry of necrobiosis).

Significance: Careful study of the stillborn monkey may give clues to the causes for and nature of processes leading to fetal demise. Study of the brains of these infants has resulted in recognition of several types of pathology seen in the human. The absence of data with regard to the circumstances of fetal compromise has prevented knowledge of pathogenesis of these lesions.

Proposed Course of Project: Postmortem studies of the stillborn fetuses will continue. An attempt will be made to correlate the facts known about the type of fetal death with patterns of pathology seen in the brains of animals falling within experimental procedures.

Publications:

Myers, R. E., Cystic Brain Alteration After Incomplete Placental Abruption in Monkey, Accepted for publication in Arch. of Neurol., 1969.

Serial No. NDS(I) 66-LPP 1386
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental placental insufficiency in the Rhesus monkey.

Previous Serial Number: Same

Prinicpal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Donald Cheek

Cooperating Units: Department of Pediatrics, Johns Hopkins University.

Man Years:

Total:	1.4
Professional:	.4
Other:	1.0

Project Description:

Objectives: 1. The effects of ligation of the fetal placental vessels upon placental morphology. 2. The relationship between placental insufficiency and fetal dismaturity. 3. The relation between placental insufficiency and brain damage.

Methods Employed: Monkeys at different gestational ages are subjected to hysterotomy. The fetal vessels bridging the space between the primary and the secondary placental cakes are identified and doubly ligated so that the secondary placenta is completely devascularized. Incisions are closed and the fetus brought to term. On cesarean section at term the gross and microscopic placental morphology is described. The infant is weighed at birth and its various organs weighed with precision after dissection. Weighed samples of the various internal organs, including muscle and bone, are sent to the Department of Pediatrics, Johns Hopkins Hospital, for chemical analysis. The placenta is sent to Sinai Hospital in Baltimore for morphological and histopathological description.

Major Findings: 1. Placental vessel ligation results in considerable alteration in the gross and microscopic morphology

of the secondary placenta. These changes have been described in a paper submitted for publication.

2. Dismaturity of the babies was seen in many cases. The greatest "small-for-dateness" observed exhibited a birth weight of only 55% of average for the newborn Rhesus monkey. Other animals with lesser degrees of pathological change of the placenta showed lesser degrees of gross retardation. This study is still under progress and more precise quantitative data will be in the offing.

3. Only one animal of the series so far has exhibited neurological abnormalities which consisted of extensor hypertonus and disordered use of the hind extremities with some clumsiness in the hands. This animal, despite 6 months survival, failed to develop normal gait. On examination of the brain there was evidence for astrocytic scarring in the white matter in the predilection zones for perivenular hemorrhage and periventricular leukomalacia which included the white matter in the parasaggital region. In addition, the subependymal astrocytes exhibited a curious hypertrophy almost to the extent of producing a mass lesion in both occipital lobes with less striking such abnormalities throughout the third and lateral ventricles and in the aqueduct of sylvius. This animal failed to exhibit pathological changes in the predilection zones for damage in animals subjected to acute asphyxia.

Significance: The findings relate to the problems of fetal dismaturity, to placental pathology in relation to alteration of fetal circulation and to brain damage in the perinatal period.

Proposed Course of Project: More cases are required in the monkey to complete studies both in terms of collection of data on dismaturity of the fetus and particularly in relation to biochemical changes and changes in cell number in relation to these procedures.

Publications:

Myers, R. E., and Fujikura, T.: Placental changes after experimental abruptio and fetal vessel ligation of Rhesus monkey placenta. Amer. J. Obstet. Gynec., 100; 846-851, 1968.

Serial No. NDS(I) 66-LPP 1387
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental placental abruption in the rhesus monkey and its relation to brain damage.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	2.1
Other:	.9

Project Description:

Objectives: To develop a model of abruptio placenta in experimental animal and to study the resultant brain pathology.

Methods Employed: Monkeys at various gestational ages are subjected to hysterotomy. Abruption of varying proportions of the placenta is produced by finger dissection. Incisions are closed and the animal is brought to term. At term, placental specimens are carefully studied for morphological changes and the fetus evaluated clinically and pathologically for brain damage.

Major Findings: In the exploratory studies thus far completed, abruptions were so extensive as to be incompatible with fetal survival in utero: In the first four animals studied, abruption of forty to fifty per cent of the total placental surface was produced. Fetal deaths followed in all four instances.

Significance: The proportion of the total placental surface which must be abrupted to produce brain damage of various types or to produce demise of the fetus can be determined at different gestational ages by using the techniques explored in the present experiment. These studies are important

to understanding of the problem of spontaneous placental abruption in relation to fetal mortality and morbidity.

Proposed Course of Project: During the past year the number of primate pregnancies available did not admit of further extension of these studies on experimental abruptio placenta. It is hoped during the coming year the number of pregnancies may be sufficient to admit further extension of these studies.

Publications: None.

Serial No. NDS(I) 66-LPP 1388
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Perinatal asphyxia in the monkey and its CNS consequences.

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.4
Professional:	.4
Other:	1.0

Project Description:

Objectives: To determine the patterns of clinical alteration and neuropathological change produced by asphyxiation at the time of birth. To attempt to correlate the patterns of neuropathological change produced with the functional changes occurring in the fetus during asphyxiation.

Methods Employed: Monkey fetuses are delivered by cesarean section at term and subjected to asphyxiation prior to first gasp by the placement of a rubber sac over the head. The lengths of asphyxiation are of predetermined length or are determined by the degree of functional deterioration produced in the fetus. An effort was made to produce the maximum degree of damage compatible with survival. Studies are carried out on the cardiovascular, blood chemical (acid base status), blood gases, pH, lactate, etc., and respiratory changes of the fetus during and after asphyxiation. Resuscitation is carried out using positive pressure oxygen ventilation.

Major Findings: With the varying lengths of asphyxiation there were varying degrees of brain damage clinically and neuropathologically apparent. The most common clinical alteration was sensory loss in the extremities and over the head of varying degrees, alterations in sucking and swallowing, alterations in the voice, and varying degrees of ataxia and clumsiness.

In some instances atrophic or traumatic sores occurred over the face and head. With more severe damage the animals exhibited changes in postural tone and changes in use of extremities with the most severe instances showing almost total loss of use of all four extremities and severe degrees of flexor hypertonus in all four extremities. Pathologically, destructive changes were found in nuclei of predilection in the brainstem and in posterior ventral and lateral ventral nuclei of the thalamus and infrequently in putamen.

In the animals showing more severe involvement there was extension of damage to increasingly larger amounts of tegmentum of the midbrain, pons and medulla, with involvement also of trigeminal and facial motor nuclei, involvement of the intermediate and ventral portions of the gray matter of the spinal cord, particularly in the lumbar region, and also extension of the destructive process in the thalamus to include progressively larger portions until this structure was literally destroyed except for small nuclear masses medially located. In general, the type and extent of neuropathological alteration could be correlated with parameters of functional alteration in the status of the fetus occurring during the asphyxial process. For example, hemorrhagic changes within the distribution pattern occurred only in instances of profound and prolonged drop in blood pressure with restitution of cardiovascular system status and survival for a period of time. Less commonly, other patterns of neuropathological change were seen such as laminar necrosis, focal cortical atrophy with ulegyria, etc. These changes were seen respectively under circumstances of severe fetal acidosis and of elevated venous pressure in relation to the asphyxial process.

Significance: The intent is to reproduce the patterns of neuropathological change seen in cases of human cerebral palsy and mental retardation. To a good extent the study has been fruitful in this regard. More important still is the effort to determine the critical parameters involved in the functional deterioration of the fetus during asphyxial insult and to relate these changes to the patterns of neuropathological change produced. Finally, efforts are being made to determine techniques through which parameters of functional deterioration of the fetus may be corrected during the asphyxial insult.

Proposed Course of Project: We hope to extend the number of physiological and blood chemical changes which are measured in relation to the asphyxial process. Efforts will be made to artificially produce alterations in functional status of the various factors determined to be important in order to more

clearly define the pathogenesis of brain damage.

Publications::

Myers, R. E.: The clinical and pathological effects of asphyxiation in the fetal rhesus monkey. In:"Diagnosis and Treatment of Fetal Disorders" K. Adamsons, Ed. Springer-Verlag, New York, pp. 226-249, 1968.

Serial No. NDS(I) 66-LPP 1389
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental hydranencephaly in the monkey.
Previous Serial Number: Same
Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	.7
Other:	.6

Project Description:

Objectives: To experimentally produce hydranencephaly in the monkey.

Methods Employed: One theory suggests that obstruction to blood flow in the major vessels of the neck plays a major role in the pathogenesis of hydranencephaly in the human. In the present study monkey fetuses are surgically removed from the uterus, preserving umbilical circulation. The fetuses are subjected to bilateral ligation of carotid arteries and jugular veins. They are then replaced in the uterus and the incisions closed. On cesarean delivery later at term the fetuses are carefully evaluated for signs of neurological abnormalities. The brain specimens are removed and studied for pathological changes.

Major Findings: (1) Hydranencephaly may result from such vascular ligation particularly when carried out during earlier gestational ages.

(2) Bilateral cerebral infarction may result in the areas of supply of the anterior and middle cerebral arteries associated with tissue collapse and the formation of extensive tissue scars.

(3) Cerebral dysgenesis may be seen involving overall distortions in brain shape and in convolutional patterning. Such brains may be of normal weight and exhibit no focal lesions.

(4) Bilateral carotid artery and jugular vein ligation may produce no effect on the brain particularly when ligation is carried out during later stages of gestation.

(5) Fetal death may result as a consequence of the vascular ligation combined with the surgical assault on the fetus. Primary fetal death occurred in approximately 46% of all cases studied in the present series.

Significance: Knowledge of the mechanism of production of hydranencephaly in the monkey will greatly elucidate the pathogenesis of hydranencephaly in the human.

Proposed Course of Project: Further studies are required to determine the etiological factors underlying hydranencephaly. At present it appears an insult beyond vascular ligation is required for experimental production of hydranencephaly.

Publications:

R. E. Myers, Brain pathology following fetal vascular occlusion: an experimental study. Invest. Ophthal. 8: 41-50, 1969

L. von Sallmann, The effect of intrauterine surgical procedures on the development of the primate eye. Invest. Ophthal. 8: 51-60, 1969.

Serial No. NDS(I) 66-LPP 1392
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Nembutal prophylaxis of brain damage.
Previous Serial Number: Same
Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Geoffrey S. Dawes
Dr. Forrester Cochburn
Dr. Benjamin B. Ross
Dr. L. Stanley James
Dr. Salha Daniels

Cooperating Units: Nuffield Institute for Medical Research, Oxford, England; University of Oregon School of Medicine, Portland, Oregon; Columbia Presbyterian Medical Center, New York, New York

Man Years:

Total:	1.3
Professional:	.4
Other:	.9

Project Description:

Objectives: In prior studies within the Laboratory both local and general anesthetics were used for surgical delivery of the fetus. Animals delivered under nembutal were more tolerant of asphyxiation than were animals delivered under local in the length of asphyxia sustained prior to deterioration of functional indices of the fetus. It was not uncommon, for example, for fetuses delivered under nembutal to sustain up to fifty heart beats per minute for as long as thirty minutes of asphyxiation whereas animals delivered under local anesthesia regularly experienced cardiac standstill after only fifteen to twenty minutes of asphyxiation. The present study seeks to further investigate the significance of barbiturates in the prophylaxis of asphyxial brain damage.

Methods Employed: Two series of animals are delivered, one under local and one under nembutal anesthesia. The animals of both series are asphyxiated for a specified length of time, measurements of various functional parameters of the fetus being

carried on during and after asphyxiation. The animals are resuscitated with partial pressure oxygen ventilation and observed clinically for the first two weeks of life. The animals are then sacrificed and studied neuropathologically.

Major Findings: With the animals asphyxiated twelve and a half minutes, all animals delivered under nembutal general anesthesia exhibited either little or no evidence for clinical abnormalities and on neuropathological examination showed evidence for only very minimal or no pathological change in the brain. All animals delivered under local anesthesia exhibited evidence of clinical neurological deficit of moderate to severe degree and on pathological examination revealed marked pathological changes in the typical distribution within the brainstem. Other animals delivered under local anesthesia but nembutalized on delivery prior to asphyxiation responded in a fashion intermediate to those determined above. It is inferred that adverse factors act producing fetal deterioration in utero prior to delivery. This deteriorated status of the fetus on delivery under local anesthesia results in a higher incidence of brain damage and death when challenged with acute asphyxia. Nembutal somehow prevents the development of fetal compromise. In addition, nembutal anesthesia produces a lowering of the metabolism of CNS tissue of the fetus resulting in a mitigation of the effects of acute asphyxia as measured both clinically and neuropathologically.

Significance: These studies are important for the problem of parturition and brain damage in the human.

Proposed Course of Project: Project completed.

Publications:

F. Cockburn, S. S. Daniel, G. S. Dawes, L. S. James, R. E. Myers, W. Niemann, H. Rodriguez de Curet, and B. B. Ross. The effect of pentobarbital anesthesia on resuscitation and brain damage in fetal rhesus monkeys asphyxiated on delivery. J. of Pediatrics (In press) 1969.

Serial No. NDS(I) 66-LPP 1396
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Neuropathology of Lead Encephalopathy in the Rhesus Monkey

Previous Serial Number: Same

Principal Investigator: Dr. A. W. Brann, Jr.

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: .5
Professional: .1
Other: .4

Project Description:

Objectives: Study effects of chronic lead ingestation on central nervous system of the monkey.

Methods Employed: Monkeys less than one month of age are given lead chloride in their milk with increasing dosage, attempting to stay under amount producing acute lead encephalopathy.

Major Findings: No major alterations in the clinical state of the nervous system occurred except for occasional episodes of acute lead encephalopathy. These subsided with no residual deficits on discontinuance of the lead. The animals have not been sacrificed as yet.

Significance: This study was initiated with the suspicion that in man some cases of mental retardation result from chronic lead ingestation unassociated with acute lead encephalopathy.

Proposed Course of Project: The study is being concluded this year. The animals will be sacrificed to study effects on central nervous system.

Publications: None

Serial No. NDS(I) 66-LPP 1397
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Comparative Studies of Ascending Spinal Projections

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Mr. Jorge Camuñas - Student

Cooperating Units: None

Man Years:
Total: .6
Professional: .2
Other: .4

Project Description:

Objectives: 1) To define patterns of central nervous system organization in vertebrates. 2) To make a survey of ascending spinal projections in several species from each vertebrate class.

Methods Employed: The animals are sacrificed 7 - 30 days after hemisection of the spinal cord, or ablation of the dorsal column nuclei. The brains are then processed according to the method of Nauta. The silver impregnated degenerating axons are identified and reconstructions are made of the course and termination of the degenrated fibers.

This year was spent mostly preparing manuscripts for publication.

Major Findings: Earlier reports have dealt with the ascending spinal projections in the Tegu lizard, the frog, the turtle, and the snake. These studies revealed a component of the dorsal funiculus that projected

directly to the cerebellum. Since there was no evidence for a spino-cerebellar component in the dorsal funiculus of mammals, lesions were placed in the dorsal column nuclei of eight cats in order to explore this issue further. This study resulted in the discovery of a pathway from the nuclei gracilis and cuneatus to the dorsal accessory olive (which in turn is known to project to the cerebellum). Specifically, degenerating fibers can be traced ventrally from the lesion as dorsal internal arcuate fibers. After crossing the midline, these fibers turn slightly rostrally and laterally before entering the inferior olivary nucleus between the various lamina of the nucleus. From here, fibers enter the dorsal accessory nucleus in which profuse pericellular terminal degeneration is observed. This fiber system is tentatively interpreted as a link between the dorsal columns and the climbing fiber afferents to the anterior lobe of the cerebellum, relaying specific information from mechano-receptors in the skin and joint receptors to the cerebellum.

Significance: Comparative studies such as these are carried out in attempts to elucidate problems of human central nervous system organization by broadening our understanding of brain organization of lower vertebrate forms. These studies are the first comprehensive studies of this kind using the Nauta method. The findings in the lizard and the frog, are, with few exceptions, similar to those observations made in all mammals so far examined. The discovery of the dorsal spino-cerebellar tract in the dorsal funiculus may represent a more primitive arrangement. A phylogenetic trend in the spino-olivary systems appears at this stage to be related to the increased role of the dorsal funicular system and associated structures in higher primates. It is noteworthy that attempts to demonstrate the classical ventral spino-olivary tract (VSOT) in chimpanzee and man have remained unsuccessful, although the connection is quite conspicuous in the rhesus monkey and other mammals examined. If the dorsal spino-olivary system (DSOS) is found to be concerned with more specific information than the VSOT, then it would be reasonable to expect that the DSOS is the vehicle from the spinal input to the dorsal accessory olive in chimpanzee and man. In general, however, a greater number of species of each vertebrate class must be examined before the generality of these observations can be ascertained.

Proposed Course of the Project: 1) This project is completed.

Publications:

ARTICLES IN A PERIODICAL:

Ebbesson, S. O. E.: A connection between the dorsal column nuclei and the dorsal accessory olive. Brain Research, 8: 393-397, 1968.

Ebbesson, S. O. E., and Camuñas, J.: Ascending spinal projections in the bull frog (Rana catesbiana). Brain, Behavior and Evolution (In press).

Ebbesson, S. O. E.: Brain stem afferents from the spinal cord in a sample of reptilian and amphibian species. In: Annals of the N. Y. Acad. Sc. (In press).

Serial No. NDS(I) 66-LPP 1398
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of neurohistological methods

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Kalman Rubinson
Mrs. Michie Vane and Miss Lolyn López - BLTs.

Cooperating Units: Walter Reed Army Institute of Research

Man Years:
Total: .4
Professional: .2
Other: .2

Project Description:

Objectives: 1) To develop more reliable neurohistological methods for the demonstration of degenerating axons, boutons terminaux, and neurons undergoing retrograde chromatolysis. 2) To improve existing methods for the demonstration of these neuronal structures, and to adapt existing neurohistological techniques for brains of submammalian vertebrates.

Methods Employed: The animals are sacrificed 1-30 days after enucleation of one eye and the brains are fixed in different fixatives. Variants of the Nauta method for the selective silver impregnation of degenerating axons are systematically investigated for improvement of staining characteristics and dependability. Investigations are underway for the development of a modification of this technique for paraffin imbedded material. The feasibility of employing fluorescent dyes for the demonstration of degenerating neurons and axons is currently being investigated.

Symposium: In addition to the research carried out in the laboratory this year, considerable amount of time was spent organizing a conference on neuroanatomical methodology. The symposium was held in San Juan, Puerto Rico during 5-8 January, 1969.

Major Findings: Modifications of the Nauta method have been developed

for the central nervous system of fishes, amphibians, and reptiles. Specifically noteworthy is a simple modification of Nauta's "uranyl nitrate method". The modification involves leaving the histological sections in a 0.01% potassium permanganate solution overnight instead of in a 0.05% solution for a much shorter period (45 - 240 minutes). When the higher concentration is used, much time is wasted performing trials which are not needed in the new procedure. The results with the weaker solution are consistently excellent and the modification is now used routinely in all of the comparative neuroanatomical studies in the laboratory.

Significance: Progress in neurobiology has always been related to the development of new techniques. The introduction of the methods for the selective silver impregnation of degenerating axons by Nauta has greatly widened the scope of neuroanatomical research. The adaptation of the technique for studies of submammalian vertebrates has broadened the field of comparative neurology. The capriciousness of the method, however, necessitates improvement as well as development of new techniques for the demonstration of degenerating axons and axon terminals. The preliminary findings suggest the feasibility of staining these structures by other than silver stains, namely, organic dyes. Such techniques would, hopefully, be easier and more reliable.

Proposed Course of the Project: This project is completed.

Publications:

ARTICLES IN A PERIODICAL:

Ebbesson, S. O. E., and Rubinson, K.: A simplified Nauta procedure. Physiology and Behavior (In press).

ARTICLE IN A BOOK:

Ebbesson, S. O. E.: Selective silver impregnation of degenerating axoplasm in poikilothermic vertebrates. In: "Contemporary Research Methods in Neuroanatomy", Ebbesson, S. O. E., and Nauta, W. J. H., Eds., Springer-Verlag, New York, 1969.

BOOK:

Ebbesson, S. O. E., and Nauta, W. J. H., Eds., "Contemporary Research Methods in Neuroanatomy", Springer-Verlag, New York 1969 (In press).

Serial No. NDS(I) 66-LPP 1399
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Comparative Studies of Retinal Projections

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Kalman Rubinson, Dr. John Ramsey, Dr. Boyd Campbell, Dr. William Hodos, Dr. Harvey Karten

Cooperating Units: Institute of Marine Biology, University of Puerto Rico.
Center for Neural Sciences, Indiana University.
Division of Neuropsychiatry, Walter Reed Army Institute of Research.
Department of Psychology, M.I.T.

Man Years:
Total: .7
Professional: .3
Other: .4

Project Description:

Objectives: 1) To define patterns of central nervous system organization in primitive vertebrates. 2) To make a survey of the retinal connections with the diencephalon and mesencephalon in several species from each vertebrate class.

Methods Employed: Unilateral enucleations of an eye have been made in the following species: 1) Toadfish (Opsanus tau); 2) Frog (Rana catesbiana); 3) Tegu lizard (Tupinambis nigropunctatus);4) Moray eel (Gymnothorax funebris); 5) Tiger shark (Galeocerdo cuvier); Nurse shark (Ginglymostoma cirratum); 6) Toad (Bufo marinus); and 7) Squirrel fish (Holocentrus sp.). The animals are sacrificed 5 to 70 days after surgery and the brains are then processed according to the method of Nauta. The silver impregnated degenerated axons are identified and reconstructions are made of the course and termination of the degenerated fibers.

Major Findings: The comparative study has been completed this year

with a considerable amount of time spent on manuscript preparation. The retinal projections in two teleost species have been analyzed in detail. Nauta preparations from toad fish (Opsanus tau) and moray eel (Gymnothorax funebris) having survived unilateral eye enucleation for 7 - 28 days revealed totally crossed projections to: 1) a small magnocellular, and 2) a parvocellular nucleus of the hypothalamus; 3) a few neurons in the fasciculus medialis tractus opticus; 4) a dorsomedial preoptic nucleus; 5) a pretectal nucleus which divides into a dorsal and a ventral part with the formation of medial and lateral marginal optic tracts; 6) a nucleus corticalis; 7) a laminated lateral geniculate nucleus (which could not be identified in the moray eel), and 8) the optic tectum.

Significance: This study on the retinal projections represents the first successful adaptation of the Nauta method to fish material. Behavioral and physiological studies of the teleost visual system have been confined almost exclusively to the optic tectum. The present findings suggest the need for additional studies encompassing various other structures receiving direct retinal projections.

Proposed Course of the Project: This project is completed.

Publications:

Ebbesson, S. O. E., and Ramsey, J. S.: The optic tracts in two species of sharks (Galeocerdo cuvier and Ginglymostoma cirratum). Brain Research, 8: 36-53, 1968.

Ebbesson, S. O. E.: Retinal projections in two teleost fishes (Opsanus tau and Gymnothorax funebris): An experimental study with silver impregnation methods. Brain, Behavior and Evolution, 1: 134-154, 1968.

Campbell, C.B.G., and Ebbesson, S. O. E.: The visual system of a teleost; Holocentrus reexamined. Brain, Behavior and Evolution (In press).

Ebbesson, S. O. E., and Karten, H. J.: Retinal projections in the Tegu lizard. Brain, Behavior and Evolution (In press).

Rubinson, K.: Retinal projections in Bufo marinus with observations on the time-course of degeneration. J. Anat. (In press).

Serial No. NDS(I) 66-LPP 1400
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Functional properties of cells within the juxta-striate area 18 of the rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Eugene C. Crichlow

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Physiology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico

Man Years:

Total:	.3
Professional:	.2
Other:	.1

Project Description: Project terminated.

Publications: None.

Serial No. NDS(I) 66-LPP 1403

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: A study of the prefrontal cortex and related structures.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigator: Dr. Ronald E. Myers

Cooperative Units: None

Man Years:
Total: 1.2
Professional: .6
Other: .6

Project Description:

Objectives: To investigate functional relations of the prefrontal lobe and related structures in the monkey by behavioral techniques, attempting to develop the functional boundaries of the macaque brain as contrasted to the anatomical ones.

Methods Employed: Nine monkeys were preoperatively trained on delayed response and go-no-go type visual color discrimination tasks. On completion of training, three monkeys underwent bilateral ablation of the anterior one-third of the temporal neocortex, three of the dorsomedial thalamus nuclei, and three served as controls. Two weeks after surgery the animals were retrained on the two tasks.

On completion of testing, they were further trained on simultaneous object color discrimination, pattern discrimination, pattern discrimination reversals, extinction tests, and position habit reversals.

Major Findings: Immediately after surgery, the temporal animals appeared tamer than preoperatively. Their appetites were lower, two actually losing some weight. These effects disappeared almost completely with time.

Postoperative performance on delayed response showed that neither the temporal nor thalamic removals had any effect. However, on the go-no-go task, rather marked impairments in performance were shown by the temporal monkeys,

whereas thalamic monkeys did not differ from the controls.

The temporal animals took more trials to reach criterion and made more errors than the thalamic or the normal animals in the postoperative learning of object color discrimination and pattern discrimination tasks. No differences were found between the latter two groups. Performance on pattern discrimination reversals revealed impairment by the temporal but not by the thalamic animals.

The results of extinction tests revealed no differences among the three groups. But in position habit reversals the temporals were again inferior to the other two groups who failed to show differences among themselves.

Significance: The impairments of performance exhibited by the temporal monkeys may be interpreted in part as being due to an impaired ability to inhibit already established response tendencies.

When interpreted in this way the deficit resembles the deficit described for lesions of orbitofrontal cortex. However, the lack of differences among the groups in extinction tests seems to question this interpretation. Further study is needed to elucidate these questions.

Proposed Course of the Project: Results obtained thus far require publication. Further studies will more closely compare prefrontal with temporal lesions.

Publications: None

Serial No. NDB(I) 69-LPP 1404
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effects of brain lesions on conditioned vocalization in monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: 0.3
Professional: 0.1
Other: 0.2

Project Description:

Objectives: To investigate brain mechanisms underlying conditioned vocalization in the monkey.

Methods Employed: Great difficulty has been found in conditioning the vocalization response in monkeys. Present efforts are directed to a direct comparison of rates of learning of vocalization and of bar-press behavior. Six monkeys were divided into three groups of two each. One group was trained to bar-press when a signal is present but to suppress bar-pressing when the signal is absent. A second group was trained in a similar way but the response required was vocalization. A third group was trained to turn the head up upon the signal and to suppress this irrelevant skeletal response in the absence of the signal.

After reaching a criterion, the signal conditions were reversed, i.e. the monkey who was trained to bar-press upon a signal was now trained to bar-press when the signal was absent.

Major Findings: Bar-press learning was the quickest of the three response types to be acquired. The head-up response was intermediate. No monkeys succeeded in utilizing vocalization responses to obtain rewards.

Significance: Neurology of vocalization as a subject of scientific investigation is of importance in relation to speech in man. The unexpected major finding in this project was the extreme difficulty in conditioning

vocalization. It appears that vocalization is much less under the control of the mechanism supporting instrumental conditioning.

Proposed Course of the Project: Study will be expanded to study the CNS structures associated with spontaneous non-conditioned vocalizations in the monkey.

Publications: None

Serial No. NDS(I) 66-LPP 1409
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Embryonic tooth development in the rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Bertram S. Kraus

Other Investigators: Dr. Ronald E. Myers

Man Years:

Total:	.5
Professional:	.1
Other:	.4

Project Description: Project completed.

Publications:

Kraus, B. S., Myers, R. E., Clark, G. R.: Teratogenic effects of carotid ligation on the developing dentition of the rhesus monkey. Teratology, (in press) 1969.

Serial No. NDS(I) 66-LPP 1410
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Reproduction and behavior in the ecology of the rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. John A. Morrison

Other Investigators: Dr. Emil W. Menzel, Jr.

Cooperating Units: Delta Regional Primate Research Center, Covington, La.

Man Years:
Total: 2.0
Professional: 2.0
Other: 1.0

Project Completed.

Publications: Menzel, E.W., Jr.: Primate naturalistic research and problems of early experience. Developmental Psychobiology, 1968 (in press).

Addendum

The following publications describe work done in the laboratory using rhesus monkeys from Cayo Santiago, relating to viral transmission and viral dissemination within the organism. Work was supported in part by Contract 46-63-25, with the National Institutes of Health through the Laboratory of Perinatal Physiology and the University of Puerto Rico School of Medicine.

Shah, K.V. and J.A. Morrison: Comparison of three rhesus monkey groups for antibody patterns to some viruses: absence of active Simian virus 40 transmission in the free-ranging rhesus of Cayo Santiago. Amer. J. of Epidemiology, 80: 3, 308-315, 1969.

Shah, K.V., S. Willard, R.E. Myers, and R. DiGiacomo: Experimental infection of rhesus with Simian virus 40 (SV40). Proc. Soc. Exp. Biol. and Med., (in press), 1969.

Shah, K.V. and D.M. Hess: Presence of antibodies to Simian virus 40 (SV40) T antigen in rhesus monkeys infected experimentally or naturally with SV 40. Society for Exper. Biology and Med., v128: 480-485, 1969.

Serial No. NDS(I) 66-LPP 1411
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Social behavior in enclosed primate groups.

Previous Serial Number: Same

Principal Investigator: Dr. Halsey M. Marsden

Other Investigators: Dr. Stephen Vessey
Dr. Duane Quiatt

Cooperating Units: University of Missouri, Columbia, Mo.

Man Years:
Total: 3.8
Professional: 1.0
Other: 2.8

Project Description:

Objectives: To investigate the social behavior of enclosed populations of primates, principally rhesus monkeys (Macaca mulatta) with emphasis on inter- and intra-group relations.

Methods Employed: Primarily direct observation of marked monkeys confined in enclosures on Cueva Island, La Parguera. Group structure is altered by removing and introducing animals.

Major Findings: A third study on intergroup relations within the two tunnel-connected enclosures is now complete. Frequency of agonistic interactions within each of the two groups of rhesus monkeys (Macaca mulatta) were found to vary as a function of the intergroup dominance relationship. During contact between the groups (doors of the tunnel open) frequency of agonistic interactions increased in the dominant group but decreased in the subordinate. Intergroup dominance was reversed and the effect of intergroup contact on intragroup aggression correspondingly reversed.

A fourth study determined the effect of food deprivation on intergroup behavior. Food was deprived in both groups for two days during each of five experimental periods. Frequency of agonistic interactions between groups decreased from 74% to 50% of total interactions, while the non-agonistic component of intergroup behavior correspondingly increased from 26% to 50%.

Aggression also decreased within each group during periods of food deprivation.

Proposed Course of the Project: The relationship between food deprivation and intergroup behavior will continue to be investigated. A planned study will deprive only one of the two groups of food. A second study involves the attempt to allow the two enclosed groups to have continual contact across the tunnel for long periods of time. The questions to be asked: Will fusion of the two groups into one occur? If so, what is the nature of the fusion process? If not, what effect on intergroup relations or intragroup social structure will such prolonged contact have?

An increasing emphasis will be placed on completing the analysis and reporting of information collected to this point in the project. It is expected that the project will be terminated during the next fiscal year.

Publications:

Marsden, H.M.: Agonistic behavior of young Rhesus monkeys following changes in social rank of their mothers. Animal Behaviour, 16: 38-44, 1968.

Marsden, H.M.: Behavior between two social groups of rhesus monkeys with two tunnel-connected enclosures. Folia Primatologica, 8:240-246, 1968.

Marsden, H.M.: Reversal of dominance between two groups of rhesus monkeys within two tunnel-connected enclosures. Second International Congress of Primatology, Proceedings, (in press).

Marsden,H.M. and S.H. Vessey.: Adoption of an infant green monkey within a social group. Communications in Behavioral Biology,(in press).

Marsden, H.M.: Intragroup aggression during contact between two enclosed groups of rhesus monkeys. American Zoologist, 8: 270, 1968.

Serial No. NDS(I) 66-LPP 1412
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Social behavior, reproduction and population dynamics of free-ranging rhesus monkeys at La Parguera

Previous Serial Number: Same

Principal Investigator: Dr. Stephen H. Vessey

Other Investigators: Dr. Halsey M. Marsden

Cooperating Units: None

Man Years:
Total: 3.8
Professional: 1.0
Other: 2.8

Project Description:

Objectives: To investigate free-ranging and enclosed primates with emphasis on social behavior, reproduction and population dynamics.

Methods Employed: Observation of marked animals. Selected animals are captured and withheld for various lengths of time to determine their role in inter- and intragroup social behavior.

Major Findings: 1) Population dynamics: As of 1 March 1969, the rhesus monkey population on La Cueva and Guayacan Islands has increased to 223, 24 of which are maintained in enclosures. Fifty-one births (seven in enclosures) occurred from April through November, 1968, with the usual peak in May and June. Thirty-two of 44 free-ranging infants survive to date, a mortality rate of 0.27. The total of 51 live births was distributed among 63 sexually mature females, giving a birth rare of 0.81.

2) Social dynamics: Four heterosexual bands occupy La Cueva Island, consisting of 70, 54, 28, and 6 individuals. A single group of 37 monkeys occupies Guayacan. Four males are solitary.

3) Molt: A two year study of pelage molt has been completed.

4) Infant behavior: In a one year study of three male and six female

infants born in 1967, activity of infant and mother was recorded at one minute intervals. Ventral contact with the mother decreased from 100% of one minute intervals to 50% in the first nine weeks, then linearly approached 0% by one year. Ventral contact with monkeys other than the mother was mainly with 3-year-old females, rising to 10% at three weeks and dropping to zero at 32 weeks. Average mother-infant distance increased linearly after the first eight weeks to 30 feet at the end of the first year. Sex differences became striking in the second six months when infants played more with infants of the same sex than with those of the opposite sex. Additional male infants born in 1968 are being studied to permit other comparisons between sexes.

5) Nighttime behavior: The use of an infrared telescope permitted identification of individuals at night, both active animals and members of sleeping clusters. These data, along with those of group activity and vocalization levels, are being analyzed.

6) Behavioral effects of removing and reintroducing individuals: Major conclusions: removal of seven dominant males in four groups resulted in no change in dominance of the group in relation to other groups and no change in the home range of the group. When an animal was removed the effects on the group were subtle. Quantitative data were collected showing changes in the behavior of the animal ranking just below the removed animal in several cases. When the dominant male was absent, the second ranking male moved into the center of the group. Following several weeks of increased aggression toward females, the new dominant male became active in breaking up fights and became more active in defending the group against outside threats (i.e. humans). Thus we can document changes that take place when a male assumes the role of dominant male, controling for individual variation by using the same male in two different roles.

When a removed animal was released, whether it was after a one day or a three month absence, aggression, grooming, mounting, following and vocalizing greatly increased, all involving the reintroduced animal. Removals of two weeks or more often resulted in failure of the released animal to remain with his original group. An all or none effect operated. The released animal either assumed his original rank in the group or became solitary or joined another group as a low ranking member. Females were more successful in rejoining their groups than males, probably because the latter spontaneously switch groups whereas the former never do. Finally, low ranking males and females were more likely to assume their former positions than dominant animals.

Thus, sex, rank and time-out-of-group are important variables in the social consequences of removal and reintroduction of free-ranging rhesus monkeys.

Proposed Course of the Project: Studies 4), 5), and 6) will be completed and the project terminated upon the departure of the principal investigator.

Publications: Vessey, Stephen H.: Behavior of free-ranging rhesus monkeys in the first year of life. Amer. Zool.8: 740, 1968.

Vessey, Stephen H. and John A. Morrison: Molt in free-ranging rhesus monkeys. J. Mammal. (in press)

Wilson, Andrew P. and Stephen H. Vessey: Behavior of castrated free-ranging rhesus monkeys. Folia Primat. 9: 1-14,1968.

Serial No. NDS(I) 67-LPP 1425
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effects of interdepression stimulation on the information content of interhemispheric transfer.

Previous Serial Number: None

Principal Investigator: Dr. Bruno Kohn

Cooperating Units: None

Man Years:

Total:	.4
Professional:	.4
Other:	-

Project Description: Project terminated.

Publications: None

Serial No. NDS(I) 67-LPP 1426
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Intermanual transfer of tactile discrimination learning in relation to the age at which commissure section occurs.

Previous Serial Number: None

Principal Investigator: Dr. Malcolm F. Piercy

Other Investigators: None

Cooperating Units: Department of Psychiatry, School of Medicine
University of Puerto Rico, San Juan, P. R.

Man Years:
Total: .4
Professional: .4
Other: 0

Project Description: Project completed.

Publications: None.

Serial No. NDS(I) 68-LPP 1427
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Changes in oculomotor activity following cerebral lesions.

Previous Serial Number: Same

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0
Professional: 0
Other: 0

Project Terminated.

Publications: None

Serial No. NDS(I) 67-LPP 1462
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Aggression in free-ranging rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Andrew P. Wilson

Other Investigators: None

Cooperating Units: University of California, Berkeley, California

Man Years:
Total: .9
Professional: .0
Other: .0

Project Terminated.

Publications: None

Serial No. NDS(I) 67-LPP 1463
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The temporal lobe connections in the monkey.

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To trace the extent of fiber projections from temporal lobe of the monkey (Macaca Mulatta) to other parts of the CNS.

Methods Employed: The second monkey for this project received total unilateral temporal lobe removal. After 10 days of survival period, it was sacrificed and perfused. The brain tissue will be processed with a modified Nauta-Gygax silver impregnation technique along with Nissle and Weil preparations.

Major Findings: The new material is being processed. New findings from this material is not available yet.

Significance: Anatomical study of cortico-cortical connections in monkeys has been relatively neglected. On the other hand, intensive efforts have been directed to the study of the cerebral cortex in the monkey with behavioral and neurophysiological techniques. Results from anatomical study should prove to be extremely beneficial to all investigators concerned with functions of the cerebral cortex.

Proposed Course of the Project: To continue as planned.

Publications: None

Serial No. NDS(I) 67-LPP 1464
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Biochemistry of the recovering acutely asphyxiated newborn monkey

Previous Serial Number: Same

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Ronald E. Myers
Dr. Alfred W. Brann, Jr.
Dr. Ronald DiGiacomo

Cooperating Units: None

Man Years:
Total: 1.2
Professional: .5
Other: .7

Project Description:

Objectives: The levels of glycogen in the brain and other tissues of the monkey newborn recovering from acute asphyxia are assayed.

Methods Employed: Monkey fetuses were delivered under Nembutal anesthesia by caesarean section and asphyxiated for two minutes beyond the last gasp. The newborn were sacrificed at birth, or 3, 6, 12, 24, and 48 hours later while the asphyxiated were sacrificed at the termination of the asphyxia, or 3, 6, 12, 24, 48, and 96 hours after resuscitation. Brain, heart, lung, liver, kidney, and muscle (adductor) were rapidly excised from the anesthetized newborn and frozen in a mixture of isopentane-isohexane cooled to the temperature of liquid nitrogen. The glycogen levels of these tissues were determined.

Major Findings: The glycogen data on this experiment has been completed. The normal levels of glycogen for the tissues of the monkey newborn in mg per gram wet weight are: brain 0.60 $\pm$ 0.13, heart 5.6 $\pm$ 2.5, lung 2.9 $\pm$ 0.4, liver 93.1 $\pm$ 33.2, kidney 1.14 $\pm$ 0.11, muscle (adductor) 29.2 $\pm$ 3.9. At the end of the period of asphyxia the glycogen levels of the various tissues are: brain -75%, heart -67%, lung +10%, liver +32%, kidney -52%, muscle -24%. The glycogen in all tissues except muscle returned to normal by 3 hours after the asphyxia. The muscle glycogen

was -33% at this time but returned to normal by six hours. The brain was the only tissue which continued to deposit glycogen reaching a maximum level at twelve hours after the asphyxia, when the level was +102% of the 12-hour control.

Significance: The brain alone of all organs exhibits an accumulation of glycogen maximal at 12 hours following the insult.

Proposed Course of the Project: To continue to analyze the tissue from the asphyxial newborn and normal animals and try to illustrate the mechanism of the chemical changes seen in the brain of these animals. We also propose to study the effects of the length of asphyxia on the accumulation of glycogen in the brain at twelve hours.

Publications: None

Serial No. NDS(I) 67-LPP 1465
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Neurometric Studies

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Capt. Douglas B. Tang

Cooperating Units: Walter Reed Army Institute of Research

Man Years:
Total: .2
Professional: .2
Other: .0

Project Description:

Objectives: 1) To determine the significance of size and numbers of nervous system components. 2) To develop mathematical and histological methods for such quantitative studies.

Methods Employed: During this year a systematic study of several variables encountered in quantitative studies of the central nervous system was completed with the use of the superior cervical sympathetic ganglion as a model. The ganglia were embedded in paraffin, cut serially, and the sections stained for the demonstration of neuronal nucleoli or treated according to the method of Bodian for the demonstration of axons.

Major Findings: The quantitative characterization of highly organized cell populations encountered in the nervous system and the implications for development and function continue to be a major area of interest to the neuroanatomists. Such studies pose many technical and methodological problems not the least of which is the sampling problem. Most quantitative studies have in common the fact that counting or measuring of particles must be carried out in serial histological sections of the cell aggregate under investigation. In but a few instances it is feasible to count or measure the particles in every

section, so that, by necessity, some sort of sampling scheme must be employed. This year we have prepared a manuscript which deals with the problem of sampling within a given section. The study considers the sampling precision of two sampling procedures for estimating the total number of nerve fibers exposed on cross section of a nerve trunk. Specifically, using the large ocular grid of the microscope to define and locate counting areas (sampling units), the mean square error associated with selecting the sampling units completely at random is compared, in nine populations of nerve fibers, with a version of the frequently employed systematic sampling method of "counting in every Kth sampling unit".

In the nine nerve fiber populations studied, the systematic selection of counting areas was generally superior (at least for small values of K); however, the increase in precision over random sampling was neither dramatic nor predictable. To obtain sampling errors of 10% or less, requires, for both procedures, counting of no less than 10% of the available sampling units.

Although the primary emphasis of this study was methodological, with results based on the study of but one type of nerve fiber populations (preganglionic sympathetic axons), it is felt that certain results will be of practical interest in other similar situations. In addition, it is hoped that this empirical study will motivate further investigations into the sampling errors of the various types of sampling methods employed in the quantitative study of the nervous system.

Significance: The need for more quantitative studies of the nervous system is clearly evident to the neuroanatomists, neurophysiologists, neuropathologists, and others. The lack of information in this area is mainly due to the magnitude and tediousness of such studies. Although computer analyses and automatic scanning devices are currently being developed for such studies, histological and statistical methods must first be developed. This project was initiated with this in mind.

Proposed Course of the Project: This project is completed.

Publications:

ARTICLES IN A PERIODICAL:

Ebbesson, S. O. E.: Quantitative studies of superior cervical sympathetic ganglia in a variety of primates including man. I. The ratio of preganglionic fibers to ganglionic neurons. J. Morphology 124: 117-131, 1968.

Ebbesson, S. O. E.: Quantitative studies of superior cervical sympathetic ganglia in a variety of primates including man. II. Neuronal packing density. J. Morphology 124: 181-185, 1968.

Tang, D. B., and Ebbesson, S. O. E.: A comparison of the sampling errors of a systematic sampling method with complete random sampling in the estimation of total number of nerve fibers. Anat. Rec. (In press).

Serial No. NDS(I) 67-LPP 1466
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Electron microscopic study of brain swelling after prolonged partial asphyxia.

Previous Serial Number: Same

Principal Investigators: Dr. Ronald E. Myers
Dr. William Bondareff

Other Investigators: Dr. A. W. Brann, Jr.

Cooperating Units: Northwestern University, The Medical School, Department of Anatomy.

Man Years:

Total:	1.2
Professional:	.7
Other:	.5

Project Description:

Objectives: Study intracellular and extracellular spaces in relation to acute total and prolonged partial asphyxial episodes.

Methods Employed: Fetal monkeys were subjected to periods of prolonged partial asphyxia or to acute total asphyxia. Sections of brain tissue are taken immediately at termination of procedure and fixed by a number of methods: Osmium tetroxide, freeze dry method, etc.

Major Findings: With prolonged partial asphyxia there is a considerable (greater than 200%) increase in the extracellular spaces relative to the intracellular spaces. Such findings are clear only with tissue fixed by freeze dry techniques. Fixation by gluteraldehyde perfusion,on the other hand, produces only a slight increase (30%) in the extracellular compartment of the brain. Closely similar changes occur at the end of insult with acute total asphyxia.

Other EM changes are noted following these insults. Most

significant of these is the ballooning up of mitochondria and a tendency to disaggregation of the ribosomal rosettes.

Significance: There is considerable need to correlate the ultrastructural changes following types of energy deprivation to the brain with the changes seen biochemically.

Proposed Course of Project: More animals will be done in both the control series and in the experimental series. Various degrees of swelling will be produced in an attempt to give a clearer picture of the progression of the process along with additional calculations of the brain compartments at these various stages of brain swelling. In this way the discrepancies between the intra and extracellular spaces by various fixation methods can be explored further.

Publications: None.

Serial No. NDS(I) 67-LPP 1471
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cardiovascular and blood chemical responses to acute asphyxia in the monkey fetuses of different gestational ages

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .7
Professional: .4
Other: .3

Project Description:

Objectives: To study the cardiovascular and blood chemical effects of acute asphyxia on fetuses of different gestational ages.

Methods Employed: Fetuses of different gestational ages are removed from the uterus still attached to the mother by the umbilical cord. They are placed into a bath of physiological saline maintained at body temperature. The carotid artery of one side is cannulated allowing for continuous recording of blood pressure, heart rate, and for the collection of blood samples at intervals. The fetus then sustains cord compression completely interrupting exchange between mother and fetus for predetermined lengths of time. Fetal pulmonary ventilation is prevented by rubber sacs placed over the head at delivery and prior to first breath. The blood chemical changes and changes in cardiovascular status are recorded. After cord release the fetus may recover spontaneously or it may require resuscitation. After restoration the fetus is returned to the uterus and the hysterotomy opening closed. The fetuses are brought to term and delivered by C-section. Clinical and neuropathological consequences of acute asphyxia are determined.

Major Findings: There are marked differences in response of fetuses of different gestational ages to acute asphyxia as brought about by cord compression. The younger fetus exhibits a marked tolerance to acute asphyxia with a gradual and late loss in blood pressure and a gradual and late decrease in heart rate. Also, blood chemical changes per unit time tend to be less dramatic in the younger fetus. Corresponding with these differences in response to asphyxia are marked differences in tolerance from the point of view of production of brain damage. The increment in hydrogen ion concentration per unit time is equal throughout the course of asphyxia for each animal whatever gestational age. pO_2 drops dramatically from levels of 40 mm of mercury at beginning of asphyxia to essentially zero levels by the end of 5 minutes. The major change occurs during the first 2 and 1/2 minutes. Throughout the remainder of asphyxia, there is no oxygen availability to the tissues and the animal survives on the basis of anaerobic energy transformations. The total CO_2 content of the blood throughout asphyxia remains essentially constant. pCO_2 increases from levels of 15 mm of mercury at the beginning to levels approximately 150 to 200 mm of mercury at the end of asphyxia. The increment in partial pressure of CO_2, despite the equality of the total CO_2 content, relates to the increase in hydrogen ion concentration producing an increment in pCO_2. Lactate concentration in the circulating blood at the beginning of asphyxiation is approximately two and a half milligrams per cent and increases during the first 10 minutes of asphyxia to levels of approximately 7.5 milligrams per cent and thereafter remains constant. Total buffer base drops from levels of approximately 40 to 45 at the beginning to levels of around 25 at the termination of asphyxia so that total buffer base availability has decreased by a factor of two. At the same time, base excess decreases rather dramatically during the course of asphyxia from levels of approximately -2.5 to levels of -20.

Significance: The changes in acid-base and in cardiovascular status of the fetus during acute asphyxia are of considerable interest in the study of the pathogenesis of asphyxial brain damage whether in relation to the perinatal period or in the adult. The present series of experiments attempt to determine the normal changes in these parameters during asphyxia in preparation for experimental studies where various parameters may be independently manipulated.

Proposed Course of Project: More cases are required to determine the shapes of the mean curves for sizeable populations of monkey fetuses of different gestational ages in order to have valid comparisons of the changes in relation to gestational ages.

Publications:

Myers, R. E.: The clinical and pathological effects of asphyxiation in the fetal rhesus monkey. In: "Diagnosis and Treatment of Fetal Disorders" Ed. by K. Adamsons, Springer-Verlag, New York. pp. 226-249, 1968.

Serial No. NDS(I) 67-LPP 1472
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Response patterns of units in striate cortex of the rhesus monkey

Previous Serial Number: Same

Principal Investigator: Dr. Ronald A. Cyrulnik

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .5
Professional: .5
Other: .0

Project Description:

Project terminated.

Publications: None

Serial No. NDS(I) 67-LPP 1473
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effect of social deprivation on the maternal behavior of primiparous and multiparous female rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2.0
Professional: 1.5
Other: .5

Project Completed.

Publications: Missakian, E.A.: The effects of social deprivation on the development of patterns of social behavior. The Proceedings of the Second International Congress on Primatology. S. Karger: Basel, Switzerland, 1969.

Serial No. NDS(I) 67-LPP 1474
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effect of social isolation on the reproductive behavior of male rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .3
Professional: .3
Other: .0

Project Completed.

Publications: Missakian, E.A.: The effects of social deprivation on the development of patterns of social behavior. The Proceedings of the Second International Congress of Primatology. S. Karger; Basel, Switzerland, 1969.

Missakian, E.A.: Reproductive behavior of socially deprived male rhesus monkeys (Macaca mulatta). Journal of Comparative and Physiological Psychology, (in press), 1969.

Serial No. NDS(I) 67-LPP 1475
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Circulatory stasis and its neurological sequellae.

Previous Serial Number: Same

Principal Investigators: Dr. James R. Miller
Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.7
Professional:	1.9
Other:	.8

Project Description: Project completed.

Publications:

Miller, J. R. and Myers, R. E. Neurological effects of systemic circulatory arrest in the monkey. Submitted to Arch. of Neurol., 1969.

Serial No. NDS(I) 67-LPP 1476
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The projections of the frontal lobe in the monkey

Previous Serial Number: Same

Principal Investigators: Dr. James R. Miller

Other Investigators: None

Man Years:
Total: .3
Professional: .1
Other: .2

Project Description: Project terminated.

Publications: None

Serial No. NDS(I) 67-LPP 1477
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Neural control of facial expression in the monkey.

Previous Serial Number: Same

Principal Investigators: Dr. Ronald E. Myers

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .8
Professional: .4
Other: .4

Project Description:

Objectives: To study brain areas involved in facial movements.

Methods Employed: The effects of lesions of cortical and subcortical centers on facial movements are being examined. Spontaneous and emotionally elicited movements are observed as the important indices of deficit.

Major Findings: Extensive lesions of cortex produce only slight to moderate amounts of facial weakness. Even total ablation of the motor cortex does not cause profound loss of spontaneous movements or any loss of response to strong emotional situations. Deficits similar in degree to that found with lesions of the motor cortex, have also been found with ablations of the prefrontal area and of the temporal lobe.

Significance: In humans, dissociation between volitional and emotional control of facial expression has long been noted. Although volitional activity has been related to the facial area of the motor cortex, areas which contribute to the emotionally generated movements are poorly known. In the monkey it appears, from the present work, that the motor cortex is far less

prominent in its contribution to facial movements than in the human.

The monkey utilizes facial movements, primarily, if not exclusively, in emotional responses. While cortical areas studied to date seem to have little effect upon these responses, it is expected that this investigation will provide information on the areas that do contribute to emotional facial expression.

Proposed Course of Project: Examination of the effects of cortical lesions is to be completed. Work will then be extended to subcortical areas.

Publications:

Myers, R. E.,
Neurology of social communication in primates. Proceedings of Second International Congress of Primatology (S. Karger, Basel) In Press, 1969.

Myers, R. E.: Discussion of paper by Dr. Leonard Radinsky entitled "Aspects of Carnivore Brain Evolution," In: Comparative and Evolutionary Aspects of the Vertebrate Central Nervous System. New York Academy of Sciences, New York. (In Press) 1969.

Serial No. NDS(I) 67-LPP 1478
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The neural control of hand strength in the monkey

Previous Serial Number: Same

Principal Investigators: Dr. Gilbert A. Preston

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: .6
Professional: .6
Other: .0

Project Description:

Objectives: To define the relationship between the sensory-motor cortex and distal motor function.

Methods Employed: A machine is utilized which tests a monkey's strength by having him squeeze two levers together to obtain food reward. The amount of force required can be varied by known amounts. When a monkey's maximum capability has been determined, neurosurgical procedures are performed and their effects on hand strength evaluated. Presently, the apparatus has been modified to obviate the use of proximal muscle groups by the subject.

Major Findings: Preliminary investigations with a device which did not totally exclude the use of proximal arm muscles indicate that extensive lesions in the hand motor cortex have to be made before any deficit in tests of arm and hand strength becomes apparent. Smaller lesions produced no effect on strength no matter where their location in the hand area. When effects were seen on strength there tended to be virtually total loss of power in the extremity. Data collecting with the new apparatus has not begun yet.

Significance: Little is known about the contributions of the major parts of the motor cortex to motor performance and motor strength. The present study with lesions of the motor cortex attempt to define clearly the effects of this important brain area on these parameters.

Proposed Course of Project: This project was delayed by previous investigators due to malfunction of the hand grip strength device. This device has been rebuilt and the project will move forward once again.

Publications: None

Serial No. NDS(I) 67-LPP 1479
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effect of visual information on intermanual transfer of problem solving in the monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Bruno Kohn

Other Investigators: Dr. Ronald E. Myers

Man Years:

Total:	.5
Professional:	.5
Other:	0

Project Description: Project completed.

Publications:

Kohn, B. and Myers, R. E.: Visual information and intermanual transfer of latchbox solving in commissure-sectioned monkey. Exp. Neurol 23:303-309, 1969.

Serial No. NDS(I) 68-LPP 1556
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effects of cerebral lesions on the social behavior of free-ranging rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Chester P. Swett, Jr.

Other Investigators: Dr. Ronald E. Myers
Dr. Michael Miller

Cooperating Units: None

Man Years:
Total: .4
Professional: .4
Other: .0

Project Description:

Objectives: To study the effects of brain lesions on the social behavior of free-ranging rhesus monkeys.

Methods Employed: Preoperative observations were carried out using members of Group E at Cayo Santiago, Puerto Rico. Data on social clusters, grooming, and aggression were recorded. Further preoperative observations as well as postoperative data on lesioned animals are now being summarized.

Fourteen animals have been trapped and operated:

Pinealectomies	2
Bilateral partial anterior temporal lobectomies	2
Bilateral anterior temporal lobectomies	5
Bilaterial anterior frontal lobectomies	5

Major Findings: 1. Pinealectomized animals return to their social group and appear to engage in normal behavior patterns. 2. Animals with partial anterior temporal lobectomies rejoin their group and appear to be normal. 3. Animals with anterior temporal lesions do not return to their social group but lead a solitary existence. 4. Animals from 2-5 years of age lead a solitary existence after anterior frontal lesions. 5. A yearling male with an anterior frontal lesion has rejoined his social group and shows little

deviation from normal behavior. 6. Solitary animals with frontal lesions differ from those with temporal lesions in that the frontal animals are hyperactive, more alert, and have a larger repertoire of communicative gestures.

Significance: The free-ranging monkeys of Cayo Santiago provide a unique opportunity to test the effects of brain lesions on social behavior. The results suggest that large anterior frontal and temporal lesions have a profound influence on social behavior.

Proposed Course of the Project: To determine if cingulate lesions produce significant effects upon social behavior.

Publications: None

Serial No. NDS(I) 68-LPP 1557
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Daytime sleep and rest patterns in bands of free-ranging rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Chester P. Swett, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .10
Professional: .10
Other: .0

Project Description:

Objectives: 1. To determine the amount and distribution of sleep and rest during the day in free-ranging rhesus monkeys. 2. To investigate the effects of social clusters (two or more animals within a two foot distance) on rest patterns.

Methods Employed: Two groups of rhesus monkeys were studied in order to determine the sleep and rest patterns exhibited by groups with varying geneological composition. Sleep and rest intervals were recorded and social clusters were identified. Distances between social clusters were also tabulated.

Major Findings: Rhesus monkeys sleep mainly during midday; females sleep significantly more than males. Rapid-eye-movement (REM) sleep occurs rarely. Animals usually rest with members of their geneologies. Animals orphaned during the second six months of life rest most often with adult males rather than with known relatives. Related animals of an entire geneology often rest in adjacent social clusters.

Significance: Rest is one of the commonest behavioral attributes of organisms. Yet few studies of rest patterns have been carried out on populations of free-ranging animals. The results of the present study indicate that rhesus monkeys rest and sleep during the day, but their rest is affected

by proximity of other groups, presence of nearby animals and nearness of related monkeys. The animals show great flexibility in adapting their behavior to obtain their required rest while maintaining the ability to monitor their environment. Such flexibility may also exist in other species.

Proposed Course of the Project: Further studies of resting social clusters and rest duration are now in progress.

Publications: None

Serial No. NDS(I) 68-LPP 1558
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Primate social patterns

Previous Serial Number: Same

Principal Investigator: Dr. Donald Stone Sade

Other Investigators: Mr. James D. Loy
Mr. Glenn Hausfater
Miss Judith Breuggemann

Cooperating Units: Northwestern University, Evanston, Illinois

Man Years:
Total: 2.7
Professional: 2.7
Other: 0

Project Description:

Objectives: To identify and describe the mutual constraints of interacting life cycles of rhesus monkeys (Macaca mulatta) maturing within a social group which has itself developed with minimum disturbance under free-ranging conditions.

Methods Employed: Daily records on behavior of all individuals within one social group (Cayo Santiago Group F) are analyzed using ethological and sociometric techniques. Observations on the same individuals have been carried out by the principal investigator since 1960. Life histories are constructed from the longitudinal records so obtained. Improved sampling techniques are being developed. Subsidiary problems are studied as possible.

Major Findings (1968-1969): 1) Weights obtained on many individuals and compared with their dominance status indicate that heavier monkeys are not necessarily dominant. Only among that subset of animals comprised of adult males who had matured within the group did weight correlate with dominance rank. It is suggested that high dominance rank during maturation results in a heavier adult male rather than the reverse. No one simple statement can adequately express the relations between dominance rank, genealogical relation, age, sex, and physical characteristics. Rather a set of statements (an algorithm) will be required and is being developed.

2) The predictable rise in rank of younger sisters over older sisters described in earlier reports continues to be observed. In Group F, eleven such cases are now documented. No present hypothesis adequately explains this phenomena but several hypotheses can be rejected on the basis of present evidence. The age in which rise in rank occurs is variable, ranging from one to four-and-one-half years for the younger sister. The rise in rank does not necessarily occur during the younger sister's first estrus period nor even during the mating season. Rise in rank also does not depend upon the presence of the mother, for in two genealogies younger sisters rose in rank over older sisters several years after the death of their mothers.

3) A smaller group of monkeys began to displace Group F after the dominant male of Group F left the group. We predicted that removal and retention of the dominant male of the smaller group would result in the rise in rank of Group F to its former position. The male of the smaller group was retained in a cage for six weeks. No change in inter-group dominance was observed. It is suggested that the control of inter-group dominance depends upon a variety of factors of which group size and the relative dominance of the highest ranking males are only two, and that these two operate in as yet unspecified circumstances.

4) After an adult female was seen drinking her own urine we tested it with a tape designed to indicate the presence of glucose. Many tests of her urine indicated a glucose content of over two percent. No other monkey in Group F, including her relatives, has yet shown a positive test for glucose in the urine. This female is the first case of spontaneous Diabetes mellitus known from Cayo Santiago. The female died during the food shortage which occurred during July, 1968.

5) During July, 1968, a ten-day food shortage contributed to the death of 19 monkeys. Other effects of the food shortage were also observed: young and diseased animals were most affected by the food shortage. Within the four lowest-ranking social groups on the island more females died than expected by chance. More yearling monkeys from all groups died than expected by chance. There was a significant correlation between group dominance rank and the percent of deaths within each social group, with lower-ranking groups suffering higher mortality. The following changes in social behavior were observed: the frequency of social grooming decreased significantly; the frequency of play decreased significantly; the frequency of fights decreased significantly; the frequency of passive body-contact, displacements of Group F by other social groups, and total group movements showed little change during the food shortage; the frequency of sexual interactions decreased. The reactions of the Cayo Santiago rhesus monkeys to food shortage closely parallel the reactions of rhesus monkeys on Desecheo Island, and of chacma baboons observed under famine conditions in Southern Rhodesia.

6) Loy made intensive observations on estrus behavior of Group F females. He concluded: Parous females without a surviving infant of the year are consistently among the earliest breeders. Copulations occur most frequently between 1500 and 1800 hours. The middle and high ranking males tend to be

more active in mating than the low ranking males. As age of male increases so does age of the female sexual partner. There appears to be no significant correlation between dominance rank of female and of male partners. Males of all ranks concentrate their mating activity in the central portion of a female's estrus period. The frequency of son-mother mating was significantly lower than the expected frequency. Favoritsm was observed between sexual partners on several occasions. Estrus behavior was seen both at mid-menstrual cycle and around menstruation. Average estrus cycle length was found to be 20.9 days with a standard deviation of 9.2 days and a range of six to 45 days. Pre- and post-conception estrus cycles averaged 20.1 and 21.0 days respectively. Estrus cycles of non-pregnant females during the mating season averaged 18.3 days; estrus cycles of non-pregnant females during the non-mating season (January through July) averaged 22.5 days: these means are significantly different. Average estrus period length was 5.2 days with a standard deviation of 4.6 days, a range of one to 33+ days, and mode of one day. The average length of those estrus periods marked by series-mountings was 6.2 days. The average length of estrus periods of non-pregnant females for the mating season and non-mating season were 6.4 days and 4.4 days respectively. The average number of males associating with an estrus female was 3.4 males per estrus period, with a standard deviation of 1.8 and a range of one to ten males. The average number of male associates from estrus periods of non-pregnant females varied little from mating to non-mating season. There was little difference in the average number of male associates during estrus periods of pregnant and non-pregnant females. There was no increase in number of male associates per estrus period with increase in age of female. Higher ranking females averaged more male associates than did lower ranking females. All gestating females showed post-conception estrus periods; the average number of such periods was 4.1 per female, with a standard deviation of 0.7 and a range of 3 to 5. Of thirteen gestating females, seven showed an increase in the frequency of their interactions with adult males during the final few months of pregnancy. Of the seven females who did not give birth in 1969, six showed signs of estrus cycling during the non-mating season. This suggests that free-ranging rhesus females may show reproductive cycles throughout the year, although of varying intensity. Thirty-three possible menstrual periods were recorded. These appeared as blood flows around the vulva of the female. One or more blood flows were sighted during every month of the year except September and October. This suggests that free-ranging rhesus females may menstruate at any time of the year. Ten of the 33 possible menses occurred simultaneously with estrus behavior. Two bleedings occurred just after and four bleedings just before estrus periods. There appear to be three basic patterns of estrus behavior; these correlate with active, less active, and least active breeders; these types are illustrated by the use of factor complexes.

Significance: To develop an intimate knowledge of the social order of the rhesus monkey as a model of a primate social order other than man.

Proposed Course of the Project: Continue observations as above on this long term study of Group F.

Serial No. NDS(I) 68-LPP 1558

Publications: None

Serial No. NDS(I) 68-1559
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effect of amygdalectomy on social behavior of free-ranging rhesus monkeys.

Previous Serial Number: Same

Principal Investigators: Dr. Arthur Kling
Dennis Dicks

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: Department of Psychiatry, University of Illinois at the Medical Center.

Man Years:

Total:	.6
Professional:	.4
Other:	.2

Project Description: Project Completed.

Publications:

Dicks, D., Myers, R. E., and Kling, A. Effects of Amygdalectomy on Social Behavior in the Free-Ranging Rhesus Monkey. Submitted to Science 1969.

Serial No. NDS(I) 68-LPP 1560
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effects of prolonged partial asphyxia on the fetal and neonatal nervous system of the monkey

Previous Serial Number: Same

Principal Investigators: Dr. Ronald E. Myers
Dr. Alfred W. Brann, Jr.

Other Investigators: Dr. Karlis Adamsons
Dr. Richard Beard

Cooperating Units: Columbia Presbyterian Hospital, New York
Queen Charlotte's Hospital, London

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description:

Objectives:

1. Outline the parameters under which brain swelling occurs in prolonged partial asphyxia.

2. Compare and contrast the brain pathology seen in prolonged partial asphyxia with that seen in acute total asphyxia.

3. Determine the minimum level of insult required to produce central nervous system damage.

Methods Employed: The femoral artery of term fetal monkeys was catheterized and electrocardiographic leads placed across the chest. The animals were returned to the uterus and subjected to a period of hypoxia with associated metabolic and respiratory acidosis.

Major Findings: When the fetal status has deteriorated such that the blood pH is below approximately 7.0 for as long as 2 hours, the brain is swollen at delivery. When fetuses failed to survive beyond the first day of life, examination of

the brain revealed, in addition to brain swelling: 1) focal areas of hemorrhagic cortical necrosis frequently involving the paracentral regions symmetrically. 2) generalized cortical necrosis with or without a hemorrhagic component. In the monkeys which survived the initial insult for 5-6 months, atrophic cortical sclerosis has been found along with destructive changes in the basal ganglia.

In recent animals surviving up to 3 days gross cerebral lesions have been seen involving the cortex in the same regions in the absence of gross swelling at autopsy. Thus, brain swelling appears not to be an essential ingredient for the production of the destructive cortical process.

Cortical damage with ulegyria is seen in animals sustaining asphyctic compromise with the pH ranging between 6.9 and 6.99 for 3 hours. Insults of lesser severity for the same length of time do not appear to produce such lesions. An episode of acute total asphyxia superimposed on the partial prolonged asphyxia results in more severe damage to cortex.

Significance: The findings of atrophic cortical sclerosis in animals subjected to prolonged partial asphyxia give insight into the pathogenesis of perinatal damage in man.

Proposed Course of Project: These studies are essentially completed and the work is entering into a data analysis phase.

Publications:

Myers, R. E., Beard, R., and Adamsons, K. Brain Swelling in the Newborn Rhesus Monkey Following Prolonged Partial Asphyxia, Neurology (In Press) 1969.

Myers, R. E. Models of Asphyxial Brain Damage in the Newborn Monkey. Proceedings of the Second Pan American Congress of Neurology (In Press) 1969.

Myers, R. E. Atrophic Cortical Sclerosis Associated with Status Marmoratus in a Perinatally Damaged Monkey. Submitted to Neurology.

Serial No. NDS(I) 68-LPP 1561
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Glycogen levels of the brain of juveniles recovering from circulatory stasis.

Previous Serial Number: Same

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. James R. Miller

Cooperating Units: None

Man Years:
Total: .1
Professional: .1
Other: .0

Project Description:

Objectives: To study the biochemical changes in brain and other tissues of the juvenile recovering from circulatory arrest.

Methods Employed: Monkeys are subjected to 14 minutes of systemic circulatory arrest by clamping the venae cavae and the arch of the aorta as they enter and leave the heart. Circulation is maintained in the lesser circulation. Following release of the vessels the animals are allowed to recover and are sacrificed at various times after the insult. The brain, liver, spleen, kidney and muscle (adductor) are quickly excised from the anesthetized monkey and frozen in a mixture of isopentane-isohexane cooled to the temperature of liquid nitrogen.

Major Findings: Normal glycogen levels of the juvenile monkey in mg per gram are: 0.63 $\pm$ 0.1, liver 43.9, kidney 0.47, spleen 1.72, muscle (adductor) 8.39. The glycogen contents of brain, heart, kidney, and spleen were depleted dramatically during the episode of ischemia, while that of liver and muscle were not affected. However, the surgical procedure alone produced a lesser depletion of liver, spleen, and muscle glycogen. The brain, unique of all organs studied accumulated glycogen in significant quantities with a peak concentration at twelve hours after the insult. The brain of insulted animals exhibited 125%

more glycogen than a sham-operated control animal, both at 12 hours past procedure.

Significance: This study supplies missing information regarding biochemical changes occurring during recovery of function following circulatory arrest. In many respects these animals are similar to those suffering from acute total asphyxia (Project No. 67-LPP 1464).

Proposed Course of Project: Investigation of the enzymes of the carbohydrate synthetic pathways will be studied.

Publications: None.

Serial No. NDS(I) 68-LPP 1562
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Biochemistry of the recovering monkey infant subjected to chronic partial asphyxia

Previous Serial Number: Same

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	.0

Project Description:

Objectives: Glycogen levels of brain and other tissues of the monkey newborn will be studied following an episode of chronic partial asphyxia.

Methods Employed: The term monkey infant is delivered and manipulated as outlined in Project No. 68 LPP 1560. Matched cesarean sectioned infants are used as controls. The brain, heart, lung, liver, spleen, kidney and muscle (adductor) are removed and instantly frozen in liquid nitrogen.

Major Findings: This project is just being initiated.

Significance: To learn the biochemical processes involved in the recovery of brain and other tissues after chronic partial asphyxia.

Proposed Course of Project: As described.

Publications: None

Serial No. NDS(I) 68-LPP 1563
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Biochemistry of in-vivo brain swelling in the monkey

Previous Serial Number: Same

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Harvey Shapiro

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Project terminated with departure of co-investigator.

Publications: None.

Serial No. NDS(I) 68-LPP 1564
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Metabolic factors involved in the production of hypoxic cerebral edema

During FY 1969 this project was incorporated with Serial No. NDS(I) 69-LPP 1715.

Serial No. NDS(I) 68-LPP 1565
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Prolonged hypoxia and symmetrical white matter necrosis in adult monkey

Previous Serial Number: Same

Principal Investigator: Dr. Harvey M. Shapiro

Other Investigators: Dr. Donald E. Myers

Cooperating Units: None

Man Years:

Total:	2.3
Professional:	1.2
Other	1.1

Project Description:

Objectives: To determine the parameters of an hypoxic insult that has produced severe symmetrical white matter necrosis with involvement of one or both divisions of the globus pallidus bilaterally.

Methods Employed: The adult monkey, under either light intravenous barbiturate or Halothane-nitrous-oxide anesthesia is exposed to a nitrogen-oxygen gas mixture that results in arterial oxygen tension varying between 17 and 23. This insult is begun after a suitable control period of mechanical volume regulated respiration, which is then maintained at the control constant for the remainder of the experimental insult. The duration of this insult is varied and the animal's mean blood pressure is maintained above a mean value of 50mm Hg. The pH, pCO_2, pO2, and base excess are monitored frequently. Continuous recordings of the systemic arterial and venous pressures and the electrocardiogram are taken. The EEG is occasionally monitored. These measurements are continued throughout control, insult, and recovery periods. Those animals surviving three or more days are examined neurologically and a gross and neuropathological study of the brain and spinal cord are performed.

Major Findings: The production of symmetrical white matter and basal ganglia lesions is of rare occurrence in the monkey. It appears that the duration of the metabolic acidosis induced by the hypoxia is a prime factor in the production of the above pathology. Treatment with large doses

of bicarbonate concomitant to the hypoxic insult may preclude the development of the lesions. As the hypoxic model has become more stable with increasing experience, incidence of hypotension has decreased as has the occurrence of white matter necrosis. One likely etiological factor in this pattern is the arterial hypotension.

Significance: These studies have established that a model for symmetrical lesions in the white matter and basal ganglia is feasible. This will allow further exploration of a lesion complex in the primate that is similar to that seen in humans.

Proposed Course of the Project: Additional animals will be studied under the above conditions to more clearly define the roles of hypoxia, acid-base disequilibrium, and systemic blood pressure in the production of this unique lesion.

Publications: None

Serial No. NDS(I) 68-LPP 1566
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Functions of the anterior corpus callosum in the monkey.

Previous Serial Number: None

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: To determine the functions of the anterior portion of the corpus callosum in monkey.

Methods Employed: Monkeys underwent midline section of corpus callosum, anterior commissure, and optic chiasm. Others received midline section of the optic chiasm alone.

Following surgery, all monkeys were taught a delayed response, go-no-go visual color discrimination and delayed alternation tasks with one eye occluded and the arm contralateral to the occluded eye restrained. Tests of transfer of training to the opposite eye and arm combination were then carried out.

Major Findings: The forebrain commissure sectioned monkeys showed fair transfer of delayed response and good transfer of delayed alternation. There was failure of transfer of the go-no-go visual discrimination.

Significance: It was anticipated that monkeys with the forebrain commissures sectioned would show lack of transfer of training on the delayed response and the delayed alternation tasks as well as on the go-no-go discrimination.

Proposed Course of the Project: The present results require a modification of thinking with regards to the neurology of the delayed response and delayed

alternation performances. Commissural lesions will be extended to subcortical systems.

Publications: None

Serial No. NDS(I) 68-1567
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30 1969

Project Title: Social organization of a large group of free-ranging monkeys as reflected in their grooming.

Previous Serial Number: Same

Principal Investigator: Dr. Margaret Varley

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .2
Professional: .2
Other: .0

Project Description:

Objectives: To describe the social structure of a large group (250 monkeys) of free-ranging rhesus monkeys on Cayo Santiago as revealed in their choice of grooming partners. This structure will be compared with that of another large group on the island, which has been described by observers as tighter and less diffuse in its organization. To explore, when possible, this structure with the dominance hierarchy of the troop.

Methods Employed: Field observation of the monkeys of this group. An attempt is made to identify the monkeys involved in every instance of grooming which is seen. The family relations of each monkey born into the group has been plotted and the data are analyzed with respect to whether the grooming partners belong to the same (familial) or different (cross-geneological) families. Because grooming reflects some degree of association or intimacy between monkeys, a picture can be drawn from the cross-geneological data of direct connections and linkages among the families of this group.

Further analysis of the data will be made in terms of age, sex, and kind of family relationship.

Major Findings: The group was observed over 400 hours in an eleven month period. 2300 instances of grooming were recorded; approximately 300 of these instances yielded cross-geneological information.

In a preliminary analysis of these data it was found that:

(1) The percentage of the total grooming which was non-familial was slightly over 30, a proportion roughly similar to that found in two other bands on the island.

(2) By devising a 26 by 26 matrix (the troop was made up of 26 families, plus males born into other groups), the troop can be arbitrarily divided into two groups. These groups are not separated but differ from one another in the relative number of interconnections between families. Fifteen of the 26 families, comprising 141 of the 208 monkeys born into Group C, have a higher degree of interrelatedness as measured by interfamilial grooming than do the remaining 11 families (57 monkeys).

(3) The simplest scheme for describing the interrelations between those 15 families is that of interlocking triangles which can be laid out on a two-dimensional plane. Thus there were no subgroups isolated from the rest. Any arbitrarily chosen subgroup (triangle of families) is connected at several other points to other such subgroups. In this way, it is postulated, the group was able to cluster functionally and selectively and still remain cohesive.

The remaining 57 monkeys seemed to have selective bonds throughout this network and with one another, but probably of weaker strength. They did not form a distinct subgroup.

Significance: The study has shown one possibility of social organization of a large group of monkeys which gives its members opportunities for small group formation and yet is very rissitant to group splits and maintains itself by certain criteria, as a single "community." It is of interest, therefore, to compare it with another type of organization of a large group on the island, which, while seeming to be cohesive, has and may be in the process of splitting. The particular organizations of other large macaque groups have not been described. However, Goodall has reported a loose kind of organization among a group of 150 chimpanzees. The superficial description of this group of apes resembles former casual descriptions of Group C.

Proposed Course of the Project: The group of monkeys is presently being removed from Cayo Santiago; data collection is complete. Analysis of the data will proceed in the coming year.

Publications: None

Serial No. NDS(I) 68-LPP 1568
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of aggressive response in a rhesus monkey.

Previous Serial Number: Same

Principal Investigator: Dr. Margaret Varley

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .2
Professional: .2
Other: .0

Project Description:

Major Findings: Three of the four infants which were born were observed for a total of 100 hours through the first four months of life, with particular attention to the appearance of gestures and vocalizations. The observations are being used in current observations of free-ranging monkeys and their young.

The group which produced no young were studied for the appearance of sexual behavior between a mother and her late adolescent son. Mating between mothers and sons is reportedly rare in the field. Although much of the behavior which was observed over 40 days may be termed aberrant, it resulted in pregnancy of the female. Observations suggest that in this pair interference in the usual course of the copulatory sequence was attributable to the behavior of the son.

Proposed Course of the Project: Project terminated with change of duty station to Punta Santiago, Puerto Rico.

Publications: None

Serial No. NDS(I) 68-LPP 1569
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The neuroanatomical basis of reproductive activity in male rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Elizabeth A. Missakian
Luis del Rio

Cooperating Units: None

Man Years:
Total: .2
Professional: .2
Other: -

Project Description:

Objectives: To determine the effect of specific neuroanatomical lesions on the reproductive behavior of male rhesus monkeys.

Methods Employed: Preoperative measures of the reproductive behavior of adult feral-reared male monkeys are recorded. Each male is mated a total of 10 times, the initial 2 hours of each exposure being intensively observed. The following behavioral measures are recorded: male grooming, female grooming, male grooming duration, female grooming duration, male aggression, male threat, mount, masturbation, and mount attempt. Following the completion of the preoperative exposures, brain lesions are made and sufficient recovery time is allotted prior to the initiation of the postoperative mating exposures. Ten postoperative matings are observed for each male monkey. The exact same measures are employed to record the postoperative exposures as were used for the preoperative matings.

Major Findings: Bilateral removal of the amygdala, anterior temporal cortex, or olfactory nerves fails individually to alter scores on measures of male grooming, female grooming, male grooming duration, female grooming duration, male aggression,

Serial No. NDS(I) 68-LPP 1570
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effect of prolonged social experience on the reproductive behavior of laboratory-reared male rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Elizabeth Missakian

Other Investigators: None

Cooperating Units: None

Project Terminated.

Publications: None

Serial No. NDS(I) 68-1571
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Internal social organization in a large group of free-ranging rhesus monkeys.

Previous Serial Number: Same

Principal Investigator: Dr. Elizabeth A. Missakian

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.0
Professional: .9
Other: .1

Project Description:

Objectives: To study the internal group organization, as reflected by grooming relationships, of a large group of free-ranging rhesus monkeys on Cayo Santiago.

Methods Employed: From January, 1968, through August, 1968, behavioral observations were scheduled for eight hours per day, two days per week. From September, 1968 to present, observations are made on a daily basis totaling an average of 30 hours per week. Special emphasis is placed on recording: (1) instances of grooming between individuals in the group; (2) instances of close spatial proximity between group members, and (3) instances of dominance relations between group members. Data is to be analyzed with respect to age, sex, familial association and parity status. The primary indicant of group organization or structure is grooming.

Major Findings: Matrices based on measures of grooming, dominance relations and spatial proximity of individuals in Group A reflect the following:

(1) Group A consists of at least four organizational units or "clusters." These units include the central group (N=173), the band (N=30), peripheral male , subgroup 1 (N=12) and peripheral male, subgroup 2 (N=10). The labeling of one unit as "central" does not imply any unique function or activity of members, but is based on the fact that this organizational unit is representative

of the greatest number of monkeys in Group A.

(2) Within each organizational there appears to be sub-units which are based on preferences in grooming partners. Within the central unit, such sub-units typically involve two or three different geneologies. Relatively little non-geneological grooming has been observed among members of the band. Sub-units seem to be defined on the basis of the geneology. Within each of the two groups of peripheral males, grooming relationships are restricted almost exclusively to monkeys from the same natal group.

(3) Within each organizational unit, the three dominance hierarchies based on geneology, adult males and adult females are linear. The hierarchy between units is also linear; the central group being highest in rank and peripheral male group 2 lowest.

(4) Measures of spatial proximity indicate that the central group and peripheral male subgroup 1 are spatially the closest. The band, although sometimes observed to occupy areas adjacent to the central group, most often feeds and remains in a separate section of the island. Peripheral males group 2 has not been observed to either feed or move consistently with any other unit of Group A since October, 1968. Their inclusion into the total group is based on measures of mutual defense.

Significance: Data collected on Group A will provide a basis of comparison of the social organization of groups on Cayo Santiago. The effect of experiental variables which may be peculiar to a large group may also be assessed once complete information is available on grooming, dominance and spatial relations.

Proposed Course of the Project: Behavioral observations for Group A monkeys will be continued through 1969.

Publications: None

Serial No. NDS(I) 68-LPP 1572
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Role of tectal commissures in inter-ocular transfer of learning in the pigeon

Previous Serial Number: Same

Principal Investigator: Dr. Laurence J. Stettner

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .5
Professional: .5
Other: 0

Project Description: Project terminated.

Publications: None

Serial No. NDS(I) 68-LPP 1573
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Anatomical projections of posterior and tectal commissures of the pigeon

Previous Serial Number: Same

Principal Investigator: Dr. Laurence J. Stettner

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

Total:	.5
Professional:	.5
Other:	.0

Project Description: Project terminated with departure of principal investigator.

Publications:

Myers, R. E. and Stettner, L. J.: Safe and reliable general anesthesia in birds. Physiology and Behavior 4:277, 1969.

Serial No. NDS(I) 68-LPP 1575
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Comparative Studies of the Telencephalon

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Lennart Heimer
Dr. Theodore Voneida
Dr. Frank Scalia
Dr. Boyd Campbell

Cooperating Units: Department of Psychology, M.I.T.
Department of Anatomy, Case Western Reserve University
Department of Anatomy, Downstate Medical Center, New York
Center for Neural Sciences, Indiana University

Man Years:
Total: .4
Professional: .2
Other: .2

Project Description:

Objectives: 1) To define patterns of central nervous system organization in primitive vertebrates. 2) To make a survey of the afferent and efferent fiber systems of the telencephalon in several species from each vertebrate class.

Methods Employed: Lesions of the olfactory tracts and in various portions of the telencephalon have been made in the Tegu lizard, the moray eel, and the nurse shark. The animals are sacrificed 5 to 40 days after surgery and the brains are then processed according to several modifications of the Nauta method. The silver impregnated degenerated axons are identified and reconstructions are made of the course and termination of the pathways under investigation.

Major Findings: During the year manuscripts were completed dealing with the following findings: 1) The olfactory tract was sectioned

unilaterally in four nurse sharks and one tiger shark and the animals were killed between 6 and 43 days after the operation. Whereas the axon degeneration was demonstrated best with the Nauta-Laidlaw technique after 3-4 weeks survival time, the terminal degeneration, as identified in Fink-Heimer preparations, was only seen in a 14-day specimen. The distribution of olfactory tract fibers is entirely ipsilateral to the lesion, the majority terminating near the olfactory peduncle. Profuse terminal degeneration is observed in a nucleus at the base of the olfactory peduncle and in the lateral olfactory area (Johnston '11). Some terminal degeneration is also seen rostrally, near the surface, in the dorsolateral quadrant of the hemisphere. Caudally the distribution of terminal degeneration in the lateral olfactory area gradually shifts ventrally and medially and extends as far as the caudal portion of the area superficiale basalis (Johnston '11), where some degenerating fibers reach the midline.

2) In order to study the pallial commissural systems in the lizard, unilateral lesions of various sizes were made in telencephalic structures of 23 animals. Hemispherectomy cases revealed that fiber degeneration in the cortical mantle was confined to the medial half. The piriform cortex, pallial thickening, and general cortex (Curwen '37) were free of degeneration. Terminal degeneration was observed in the following areas: 1) among the dendrites of the neurons in the medial cortex. 2) around the perikarya and dendrites of the dorsal part of the hyppocampal cortex; 3) and in the ventral cell plate of Unger ('06).

Significance: The Nauta method provides us for the first time with a satisfactory tool for the tracing of thinly myelinated and nonmyelinated axons, and since primitive vertebrates have a great proportion of such fibers, we are now able to trace pathways that older methods failed to elucidate. The studies reported on here are the first of their kind and several more species from each vertebrate class must be examined before the significance of these observations can be discussed.

Proposed Course of the Project: This project is completed.

Publications:

Ebbesson, S. O. E., and Heimer L.: Projections of the olfactory tract fibers in the nurse shark (Ginglymostoma cirratum). Brain Research. (In press).

Ebbesson, S. O. E., and Voneida, T. J.: Pallial cytoarchitecture in the Tegu lizard (Tupinambis nigropunctatus). Brain, Behavior and Evolution. (In press).

Voneida, T. J., and Ebbesson, S. O. E.: Pallial commissures in the Tegu lizard (Tupinambis nigropunctatus). Brain, Behavior, and Evolution. (In press).

Serial No. NDS(I) 68-LPP 1576
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project: Comparative Studies of the Mesencephalon

Previous Serial Number: Same

Principal Investigator: Dr. Sven O. E. Ebbesson

Other Investigators: Dr. Kalman Rubinson
Dr. Harvey Karten
Dr. James M. Petras

Cooperating Units: Department of Psychology, M.I.T.
Department of Neurophysiology, Walter Reed Army Institute of Research

Man Years:
Total: 1.0
Professional: .7
Other: .3

Project Description:

Objectives: 1) To define patterns of central nervous system organization in vertebrates. 2) To make a survey of afferent and efferent connections of the mesencephalon in several species from each vertebrate class.

Methods Employed: The animals are sacrificed 5-30 days after lesions are made and the brains are then processed according to the method of Nauta. The silver impregnated degenerating axons and terminals are identified and reconstructions are made of the course and termination of the pathways.

Major Findings: During this year manuscripts were completed on the projections of the optic tectum in the frog and lizard. Following deep lesions of the caudal portion of the optic tectum of the frog, degeneration was traced with a modified Nauta technique to the contralateral tectal hemisphere via the tectal commissure, and to the pretectum and ipsilateral neuropil of Bellonci via a medial ascending tract. Other ascending pathways pass medial to the lateral marginal optic tract and also within the commissura transversa to reach ipsilateral and contralateral

thalamic nuclei, the suprachiasmatic hypothalamic region, and the contralateral mesencephalic tegmentum. Descending tracts project, ipsilaterally, to reticular formation, interpeduncular nucleus, nucleus isthmi, and superior olive. Contralateral projections were to the reticular formation and the medial intermediate region of the cervical spinal cord.

More rostral lesions showed the same pattern with the addition of degeneration in the motor root of the trigeminus, the region ventrolateral to the trigeminal motor nucleus, and the descending trigeminal tract. These additional projections are interpreted as those of the trigeminal mesencephalic nucleus. The projections of the tectum in the lizard, are remarkably similar although the accepted nomenclature does not always agree, e.g., the projection to the neuropil of Bellonci in the frog is probably homologous to the projection to the ventral portion of the lateral geniculate in the lizard.

Significance: Comparative studies such as these are carried out in attempts to elucidate problems of human central nervous system organization by broadening our understanding of brain organization of lower vertebrate forms. These studies are the first comprehensive studies of their kind using the Nauta method. The findings in the frog and lizard are, with few exceptions, similar to those observations made in mammals. A larger sample of species must be examined before the significance of these observations can be ascertained.

Proposed Course of the Project: This project is completed.

Publications:

Rubinson, K.: Projections of the optic tectum in the frog. Brain, Behavior and Evolution. (In press).

Rubinson, K.: Connections of the mesencephalic nucleus of the trigeminal nerve in the frog. An experimental study with silver impregnated methods. Brain Research (In press).

Ebbesson, S. O. E., and Karten, H. J.: Projections of the optic tectum in the Tegu lizard (Tupinambis nigropunctatus). Brain, Behavior and Evolution (In press).

Serial No. NDS(I) 69-LPP 1696
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Microelectrode investigation of sensory input to the frontal cortical eye fields of the monkey.

Previous Serial Number: None

Principal Investigator: Dr. Jerome Engel, Jr.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.6
Other: 0

Project Description:

Objectives: This project is primarily intended to study the effect of peripheral visual input on the activity of individual frontal eye field (FEF) neurons, and the possible neuronal connections involved. It is also intended to study the effects of other sensory modalities on these neurons and to describe interactions of various modalities on FEF unit activity.

Methods Employed: Extracellular microelectrode recordings from FEF neurons are carried out in restrained lightly anesthetized or curarized rhesus monkeys. Diffuse and specific photic stimulation, electrical stimulation of the visual pathways, auditory stimulation, and tactile stimulation are employed to elicit unit responses. Subtle changes in unit firing patterns can be ascertained by averaging poststimulus intervals with a Computer of Average Transients.

Major Findings: This project is still in the process of development but good extracellular unit recordings have already been made with tungsten electrodes in the FEF. Unit responses to diffuse photic stimulation and electrical stimulation of visual pathways are now being investigated.

Significance: This study will provide specific information regarding the mechanisms of the initiation of eye movement, but more importantly, will add to our understanding of the action and interaction of cortical connections.

Proposed Course of the Project: When FEF unit responses to various

sensory inputs are determined in the rhesus, parallel studies will be carried out in the baboon, Papio papio, which has demonstrated a type of photosensitive epilepsy beginning specifically in the FEF. It is hoped that a comparison between normal and abnormal FEF unit responses to sensory stimuli may help to elucidate both normal and abnormal mechanisms.

Publications: None

Serial No. NDS(I) 69-LPP 1697
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effects of prolonged partial fetal asphyxia on subsequent electrical activity of the nervous system of the monkey.

Previous Serial Number: None

Principal Investigator: Dr. Jerome Engel, Jr.

Other Investigators: Dr. Alfred W. Brann, Jr.

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.6
Other: 0

Project Description:

Objectives: This study is intended to determine whether or not abnormal electroencephalographic activity is a sequela of prolonged partial fetal anoxia in monkeys and, if it is, to characterize the onset and maturation of this activity.

Methods Employed: The monkeys used are those subjected to partial anoxia by Dr. A.W.Brann, Jr., as described in Project No.68-1560. Routine electroencephalograms are being carried out on experimental and control monkeys two hours and 24 hours after delivery and then serially at intervals of one to two weeks until death or sacrifice. Metrazol activation will be performed on monkeys which show no abnormal activity by six months of age.

Major Findings: No electroencephalographic abnormalities are observed immediately after birth in monkeys which have been subjected to partial fetal anoxia resulting in blood pH above 6.9. There is some indication at this time, however, that asymmetrical biparietal spikes and spike and wave patterns develop in these monkeys after 3 to 4 months of life.

Significance: In addition to providing another parameter for the study of the effects of prolonged partial fetal anoxia on the monkey CNS, this preparation can yield information regarding the pathogenesis and development of epileptic activity in the immature brain. It is hoped that this study might lead to the development of a model for certain types of epilepsy seen in human children.

Proposed Course of the Project: More fetal asphyxias will be carried out and serial electroencephalograms will be continued as before. These animals will be eventually sacrificed and correlations will be made between electrical activity and pathological anatomy of the brains.

Publications: None

Serial No. NDS(I) 69-LPP 1698
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cortical projections through the anterior commissure.

Previous Serial Number: None

Principal Investigator: Dr. Kazuo Hara

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .1
Professional: .1
Other: .0

Project Description:

Objectives: To investigate the distinction of the cortical areas to which nerve fibres of the anterior commissure project in the monkey.

Methods Employed: Midsagittal section of the anterior commissure from below: histological examination of the brain by Heimer-Ebbeson's improved method for the selective silver impregnation of degenerating axoplasm: mapping the terminations of degenerating fibres on brain diagrams.

Major Findings: Project being initiated.

Significance: Some discrepancies among findings on the interhemispheric transfer of learning after sectioning the corpus callosum are thought to be due to whether or not the anterior commissure has been sectioned. This project seeks to provide anatomical bases for further understandings of the functional roles of the commissural fibres.

Proposed Course of the Project: To proceed according to the plan.

Publications: None

Serial No. NDS(I) 69-LPP 1699
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Functions of forebrain commissures in visual learning transfer in the monkey.

Previous Serial Number: None

Principal Investigator: Dr. Kazuo Hara

Other Investigators: Dr. Ronald E. Myers
Dr. Perry Black

Cooperating Units: Department of Neurological Surgery, The Johns Hopkins University Hospital, Baltimore, Maryland

Man Years:
Total: .4
Professional: .4
Other: .0

Project Description:

Objectives: To determine the functional roles of the corpus callosum and the anterior commissure in interhemispheric transfer of visual learning, the degrees of transfer deficits in relation to the complexity of visual stimuli and the difficulty of discrimination problems after midline section of those commissural fibres.

Methods Employed: Midsagittal sectioning of the optic chiasm, the corpus callosum and the anterior commissure: learning of simultaneous discrimination on one of brightness, color, geometric form and pattern by one eye while the other eye is occluded: transfer test of the above discrimination learning by the eye previously occluded: repetition of learning and transfer tests with the remaining stimuli in random order.

Major Findings: Project being initiated.

Significance: Though the lack of interhemispheric transfer of visual learning after midline section of commissural fibres has been observed by many investigators in the cat and the monkey, much ambiguities on the nature of those deficits still remain. The present study aims to clarify some of the issues which relate to such variables as the difficulty of discrimination problems and the complexity of stimuli as well as the degree of sophistication

of subjects and the interproblem transfer or generalization of learning task.

Proposed Course of the Project: To proceed according to the plan and also to extend the study to the transfer of discrimination reversal and serial reversal learning.

Publications:

Black, P. and Myers, R.E.: Brainstem mediation of visual perception in a higher primate. Transactions of the American Neurological Association, 1968, 93, 191-193.

Black, P. and Myers, R.E.: Behavioral studies in the commissure-sectioned chimpanzee: interhemispheric transfer of visual information. Proceedings of Second International Congress of Primatology, S. Karger, Basal. (In press) 1969.

Myers, R.E.: The corpus callosum and its functions. Bratislarske Lekarske Listy. (In press) 1969.

Serial No. NDB(I) 69-LPP 1700
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Associational cortex function in visual learning.

Previous Serial Number: None

Principal Investigator: Dr. Kazuo Hara

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: .4
Professional: .4
Other: .0

Project Description:

Objectives: To explore the roles of three sectors of association cortex in visual processes: a) prestriate circumscribed sector (areas 18 and 19), b) middle sector of the latero-ventral surface of the middle temporal gyrus (caudal halves of the area 20 and 21), c) anterior ventral sector of the middle temporal gyrus (rostal halves of the areas 20 and 21).

Methods Employed: Single or combined unilateral cortical ablations in the three areas described with split-brain preparation, i.e., midline sections of the corpus callosum, anterior commissure and the optic chiasm: initial learning of visual discrimination by one eye and relearning of the same discrimination by another eye previously occluded -- the initial learning to be assigned to either hemisphere, intact or insulted: repetition of initial learning and relearning by different eyes for discrimination reversals, conditional discrimination or learning set problems.

Major Findings: Project being initiated.

Significance: The present design of experiment permits us to pursue neuroanatomical loci for the formation of learning and the transfer of memory by comparing the saving scores of various learning tasks at their interhemispheric transfer from the intact to the injured hemisphere or vice versa among those subjects receiving different types of cortical insults.

Serial No. NDS(I) 69-LPP 1701
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effects of visual deprivation in early life on vision and visual learning in the monkey.

Previous Serial Number: None

Principal Investigator: Dr. Kazuo Hara

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .1
Professional: .1
Other: .0

Project Description:

Objectives: To observe any visual deficits, learning retardation or changes in interhemispheric transfer after visual deprivation beginning immediately after birth.

Methods Employed: Suturing the eyelids of both eyes of infant monkeys immediately after their birth; raising them with their mothers in the same cages for one year; split-brain procedures; observation of the development of visually guided behaviors at the cages with dimmed lights and the process of visual discrimination learning in an experimental apparatus with one eye opened; observation of the transfers of those visual tasks when the side of monocular vision is shifted.

Major Findings: Project being initiated.

Significance: A complete lack of exposure to light stimuli from early life provides many critical tests for the maturation of visual perception, the development of visual learning and of commissure functions in relation to these processes. Findings from this study are expected to have wide applicability for the rehabilitation of human blindness as well as for the psychological understanding of sensory deprivation and its effects.

Proposed Course of the Project: To proceed according to the plan.

Publications: None

Serial No. NDS(I) 69-LPP 1702
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Protein synthesis in monkey brain

Previous Serial Number: None

Principal Investigator: Dr. Stanley Holstein

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other	0.0

Project Description:

Objectives: To study the mechanism of protein synthesis in monkey brain tissue under control conditions as well as certain adverse conditions such as hypoxia. Also, to compare the findings in monkey brain with other tissues already studied.

Methods Employed: Isolation and preparation of polyribosomes by high speed differential centrifugation; also by density gradient centrifugation. Protein synthesis is determined by use of radioactively labelled (C-14) amino acid precursors of protein and assays of radioactive protein formed is accomplished by scintillation counting techniques.

Major Findings: The monkey in-vitro cell free system incorporates amino acids to form protein comparable to other tissues studied, such as liver. Hypoxic insult appears to decrease to some degree the amount of polyribosome necessary for amino acid incorporation. All results are preliminary.

Significance: 1) Equivalence of protein synthesizing machinery in monkey brain to those of other previously studied tissues. 2) damage to brain tissue may result in damage of the protein synthesizing machinery. Nature of mechanism of this is uncertain but early indica-

tions are that energy is a major requirement for protein synthesis.

Proposed Course of Project: Much more in the way of controlled amino acid incorporation studies is required. Also, the specific nature of mechanism of hypoxia is to be studied with the use of radioactivity labelled amino acid precursors injected intrathecally or subarachnoidally.

Publications: None.

Serial No. NDS(I) 69-LPP 1703
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The effects of rapid correction of acid-base abnormalities on the brain pathology seen following acute total asphyxia

Previous Serial Number: None

Principal Investigator: Dr. Stanley James

Other Investigators: Dr. Alfred W. Brann, Jr.
Dr. Karlis Adamsons
Dr. Ronald E. Myers

Cooperating Units: College of Physicians and Surgeons, Columbia University, Departments of Pediatrics, Obstetrics and Gynecology

Man Years:

Total:	1.0
Professional:	.5
Other:	.5

Project Description:

Objectives: To study the effects of rapid correction of acid-base abnormalities on the nervous system lesions seen following acute total asphyxia.

Methods Employed: Term rhesus monkeys were delivered by cesarean section and subjected to a period of acute total asphyxia extending to 2 minutes past the last gasp. They were immediately resuscitated in the usual fashion but with a constant infusion of THAM buffered to a pH of 8.65. The animals were allowed to survive 5 days and then sacrificed. The brains were removed for examination and study.

Major Findings: The data are not complete. The physiology and biochemistry are being analyzed by the cooperating unit. Neuropathological evaluation of the tissues is in progress at the Laboratory of Perinatal Physiology.

Significance: The study should determine the possible ameliorative or preventive effects on the CNS lesions of acute total asphyxia of the rapid correction of the acid-base abnormalities associated with acute total asphyxia. If positive effects occur with this additional therapy, a similar therapy program may be employed in the clinical setting in the labor room on newborn nursery for human infants (Dr. Brann).

Proposed Course of Project: Two more animals are needed to complete the data that has already been collected in this project. The data will be finally analyzed and prepared for publication.

Publications: None.

Serial No. NDS(I) 69-LPP 1704
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Thalamo-cortical projections in rhesus monkeys

Previous Serial Number: None

Principal Investigator: Dr. G. Jayalakshmi

Other Investigators: Dr. Ronald E. Myers

Man Years:
Total: 1.0
Professional: 0.6
Other: 0.4

Project Description:

Objectives: To elucidate and describe the overall pattern of neural connections from the thalamus to the cortex of the adult rhesus monkey.

Methods Employed: Adult rhesus monkeys underwent sectioning of the corpus callosum and anterior commissure and were allowed a one-year rest period to allow removal of these degenerating fibers. The thalamus was then removed on one side through the earlier sectioned corpus callosum. The animals were then sacrificed and the brains sectioned and stained according to the Nauta methods. Silver stained slides are studied and graded for cortical degeneration, and a plot of the quantities of degenerating fibers made on brain photographies to illustrate the pattern of thalamic projections.

Major Findings: The primary sensory areas of cortex are heavily receptive of thalamic projections while the remaining association cortex is remarkably free of such projections.

Proposed Course of Project: As described.

Publications: None.

Serial No. NDS(I) 69-LPP 1705
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Hypoglycemic brain damage in the monkey: An experimental model

Previous Serial Number: None

Principal Investigator: Dr. Kenneth J. Kahn

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.0
Other:	.3

Project Description:

Objectives: 1) To develop a model for lack of energy to the central nervous system by elimination of the substrate glucose. 2) To determine the reproducibility of such a system in creating brain damage. 3) To control other variables such as acidosis, hypercarbia, hypoxia, and hypotension which might contribute to brain damage. 4) To study the neuropathology associated with hypoglycemic brain damage and compare it to the other models which have been developed in the laboratory which decrease the energy available to the brain by ischemia, hypoxia, and anoxia. 5) To use the model for biochemical and electronmicroscopic evaluation.

Methods Employed: Priming a fasted animal with insulin to reduce liver stores of glycogen. Then giving a large dose of insulin and catheterizing femoral artery and vein and monitoring acid-base balance, blood gases, blood pressure, electrocardiogram and blood sugar levels. At the end of a predetermined time of blood sugars below 20mg% the animal is given sugar and nursing care and allowed to survive a variable period depending on the purpose of the experiment.

Major Findings: Reproducible brain damage occurs after 6 hours of hypoglycemia uncomplicated by acidosis, hypoxia, hypercarbia, or hypotension. Recovery, often incomplete, of brain function occurs

slowly over several weeks. Personality changes and sensory loss are the major permanent neurological residuals. Neuropathological changes have been observed in the cortex and corpus striatum and thalamus. The changes have been loss of neurons and gliosis. The inferior colliculus, which is involved in anoxic and ischemic brain damage, is normal.

Significance: Hypoglycemic brain damage, uncomplicated by contributing factors mentioned above, has never been studied. This model presents the biochemist with a mechanism in vivo of studying changes associated with cell death or cell recovery. Physiologic parameters of lack of glucose to the brain may be studied.

Proposed Course of Project: Models have and will be made available for biochemical evaluation and electronmicroscopic study. Added monitoring of cerebral metabolism, electroencephalograph, and cerebral impedance are beginning.

Publications: Abstract: Hypoglycemic Brain Damage in Monkey: An experimental model. Neurology 19: 317, 1969.

Serial No. NDS(I) 69-LPP 1706
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effect of hypothermia on the neuropathology seen following acute total asphyxia

Previous Serial Number: None

Principal Investigators: Dr. James Miller and Dr. Faith Miller

Other Investigators: Dr. Alfred W. Brann, Jr.
Dr. S. James
Dr. K. Adamsons
Dr. Ronald E. Myers

Cooperating Units: Tulane University School of Medicine
Department of Anatomy and
College of Physicians and Surgeons,
Columbia University, Departments of
Pediatrics and Obstetrics and Gynecology

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: To study the effects of hypothermia on prevention of the brainstem lesions seen following acute total asphyxia.

Methods Employed: Term rhesus fetal monkeys were delivered and immediately subjected to a period of acute total asphyxia extending to two minutes after the last gasp. The animals were resuscitated in the usual fashion with the additional therapy of hypothermia to temperatures ranging from 20 - 22° C. After the monkeys reached these temperatures they were taken out of the cooling media and allowed to warm on their own. The animals were then allowed to survive for 5 days and sacrificed.

Major Findings: The data is not complete. The physiology and biochemistry is being analyzed by the cooperating units.

The histological examination of the brain is presently in process at the Laboratory of Perinatal Physiology.

Significance: The effects of hypothermia on possible amelioration or prevention of brain damage produced by acute total asphyxia are being explored. If positive effects of hypothermia are seen in preventing brain damage, the possibility of applying this to the clinical setting of asphyxia in the newborn may be considered (Dr. Brann).

Proposed Course of Project: The animals required for this study have been completed. The analysis of data is in progress.

Publications: None.

Serial No. NDS(I) 69-LPP 1707
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Mother-infant-yearling social development

Previous Serial Number: None

Principal Investigators: Dr. Michael H. Miller
Dr. Duane Quiatt

Other Investigators: None

Cooperating Units: University of Illinois, Department of Psychiatry
University of Missouri, Department of Anthropology

Man Years:
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To identify and describe developing social patterns within genealogical units at the time of and after birth of the infants in a larger social group of rhesus monkeys (Macaca mulatta) under free-ranging conditions.

Methods Employed: Daily observations on selected genealogical units within one social group (Cayo Santiago Group E) are analyzed using sociometric techniques. Procedures involve coordinating checklist and field-note recordings of familial interactions. Infant socializations and changing patterns of intra-family associations are so obtained.

Major Findings: Projected findings will determine the influence of the mother's rank within the social group on patterns of development of the infant. Yearling aggression and weaning will be compared with genealogical positions within the group hierarchy. Parity is also considered to be of importance in so far as mother-infant interactions are concerned; with changes in infant-sibling behavior also expected.

Proposed Course of the Project: The immediate interests of the project will be pursued until the infants have attained the age of 4-6 months. It is possible that several intact genealogies will continue to be observed after transfer to 1/4 acre enclosures at the termination of this project period.

Publications: None

Serial No. NDS(I) 69-LPP 1708
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Adaptation of a free-ranging rhesus monkey group to artificial group fission and transplantation to a new environment.

Previous Serial Number:

Principal Investigator: Dr. John A. Morrison

Other Investigators: Dr. Emil W. Menzel, Jr.

Cooperating Units: Delata Regional Primate Research Center, Covington, La.

Man Years:
Total: .2
Professional: .2
Other: 0

Project Description:

Objectives: 1) To observe the effects on social behavior of dividing a long-existing social group of rhesus monkeys into two groups. 2) To observe the behavioral and ecological effects of moving members of a social group to a markedly different environment.

Methods Employed: Morrison and Menzel observed the Desecheo Island portion of the group, May 27-31, and visited the Cayo Santiago part June 3-7. Dr. William Draper, Acadia University, Nova Scotia, participated as an observer on both visits.

Major Findings: The Desecheo monkeys had separated into two bands with an adult male apparently dominating each band. Draper encountered the first band at the southwest corner of the island on 28 May. It contained 2-25 adults of which 12 were identified. Five identified and four unidentified females carried infants. Most of the infants were $<$ 2 weeks old and none were $>$ 6 weeks. Morrison located the other band on 29 May in the south-central part of Desecheo. Thirteen monkeys $>$ 1 year old and 4 infants $<$ 6 weeks old were present. All adults and subadults were identified.

The male leading the larger band formerly ranked second in dominance when the colony was established. The leader of the smaller band ranked third. The male that formerly led the entire group, including the Cayo

Santiago part, has been missing since February, 1967. He is possibly with a third band containing up to 10 adult females plus some subadults and juveniles. If so, the third band was not found.

This was the sixth visit to Desecheo Island since the monkeys were taken there in July, 1966, and we found it more difficult to find the monkeys than during any previous trip. This was not because of increased wildness. The monkeys were extremely docile and approachable, flight distance being as little as 2-3 m. The difficulty may have been caused by decreased mobility and decreased vocalization.

The island was exceptionally dry and vegetation was considerably depleted. Much of the abundant prickly-pear cactus was dead and withered. Grasses were depleted. Herbaceous food was scarce, with tree leaves and fruit minimal and forbs extremely few. Where formerly the single group moved constantly through a predictable route and could be intercepted reliably, the two or three bands may now have settled into areas of choicer food that we bypassed in searching.

Water did not appear to be a problem. Several animals obtained water from tree hollows. Rain falls almost every afternoon and provides temporary pools and wet leaves to lick. The total rainfall is considerably below average but enough falls sufficiently often to support the monkeys.

All animals appeared healthy and in the best physical condition since being released on Desecheo two years ago. Particularly noteworthy was the complete absence of fresh wounds. No new wounds have been seen on these monkeys since their release here.

The annual birth season at Cayo Santiago peaks in February and March and virtually ends by early May. Most of the infants on Desecheo appeared 1-2 weeks old. The mating season here has apparently changed from that at Cayo Santiago to a later annual period synchronous with the mating season of rhesus at La Parguera in southwestern Puerto Rico where the peak of reproduction occurs three months later in the year than at Cayo Santiago on Puerto Rico's east coast.

The monkeys in the larger band at Desecheo did not consist of strong family groups. Several animals were relatives of monkeys yet on Cayo Santiago. Monkeys in the smaller band included female 57 and her family and descendents of females CU and her daughter CQ although CU is missing and CQ is in the large band. Among the missing monkeys are female 80 and many of her offspring and descendents. They could have comprised the unidentified animals in the large band or could be in a third group.

Proposed Course of the Project: Completed.

Publications: None

Serial No. NDS(I) 69-LPP 1709
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Changes in nucleic acid content of adult monkey brain incident to hypoxia

Previous Serial Number: None

Principal Investigator: Dr. Robert O. Petersen

Other Investigators: Dr. Harvey M. Shapiro
Dr. Ronald E. Myers
Dr. Americo Rivera, Jr.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	2.0
Other:	0

Project Description:

Objectives: Evaluation of the changes in tissue concentration and distribution of RNA and DNA as well as the changes in the polysome population occurring in monkey brains during and after varying periods of hypoxia produced by Dr. Harvey M. Shapiro. A correlation of these changes with the concomitant changes in brain glycogen levels as determined by Dr. Americo Rivera.

Methods Employed: RNA and DNA are extracted by a modified procedure of the original Schmidt-Thannhauser protocol. Quantitation of RNA and DNA is accomplished by ultraviolet spectrophotometry using appropriate standards. Tissue fractioning is accomplished by differential centrifugation yielding a nuclear, microsomal, and S3 supernatant fractions. Nucleic acid quantitation described above is carried out on each of these fractions. Sucrose gradient centrifugation analysis is carried out characterizing the microsomal population.

Major Findings: Layering the post-mitochondriel supernatant over 2.0 molar sucrose followed by centrifugation for four hours at 200,000 x g results in the isolation of a pellet composed of the microsomal fraction. To date the major effort has been development

of this protocol which yields a product that is relatively pure as evaluated by: (1) spectral characteristics, (2) sensitivity to RNase, (3) the RNA protein composition. Control data relating cerebral cortical nucleic acid values ha ve been obtained.

Significance and Proposed Course of Project: With the development of procedures resulting in a relatively pure product that is representative of the tissue microsomal particle population, as well as reliable extraction and quantitation procedures from brain nucleic acids, the project is now evaluating the alterations in the cerebral cortical nucleic acids incident to the hypoxic insult.

Publications: None.

Serial No. NDS(I) 69-LPP 1710
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through July 30, 1969

Project Title: Changes in the nucleic acid content of adult monkey brain incident to hypoglycemia

Previous Serial Number: None

Principal Investigator: Dr. Robert O. Petersen

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Descriptions:

Objectives: Evaluation of the changes in tissue concentration and cellular distribution of RNA and DNA as well as in the changes in the polysome population occurring in monkey brain during and after a six-hour hypoglycemic insult. Correlation of these changes with alterations in the clinical status and physiological parameters evaluated by Dr. K. Kahn and also with the changes in brain glycogen levels determined by Dr. Americo Rivera.

Methods Employed: At varying time intervals during the hypoglycemia protocol as outlined by Dr. K. Kahn, animals are killed and cerebral tissue obtained for isolation of RNA and DNA. The isolated fractions are quantitated by ultraviolet spectro-photometry using appropriate standards. Tissue fractionating is accomplished by differential centrifugation and nucleic acid analysis as described above, are carried out on the nuclear, microsomal, and S-3 supernatant fractions obtained. Aliquots of cerebral tissue are used for dry weight determinations and subsequent protein quantitation. Finally, sucrose gradient centrifugation analysis of the microsomal population is accomplished on the remaining tissue.

Major Findings: No apparent alterations of the brains polysome population occur during 48 hours of fasting. During the subsequent six

nours of hypoglycemia there is a breakdown of the polysome structures evident after only three hours and complete breakdown to monosomes after six hours. Restoration of polysomal structures is not evident until 24 hours after the recovery of normal blood glucose levels. Complete restoration has been detected at 60 hours. No consistent changes in tissue RNA, DNA, or protein concentrations have been found to date.

Significance: The finding that energy deprivation results in polysome breakdown indicates the necessity of adequate energy stores for maintenance of structural integrity of the polysome particles in vivo. As protein synthesis takes place on the polysomal structures, the breakdown of these particles should result in a cessation of protein synthesis. Finally, correlating the findings supplied by Dr. Rivera's studies on brain glycogen, it is most interesting that 12 hours after restoration of normal blood glucose levels, a time when the brain shows glycogen levels above control values, the polysomal structures have not yet reappeared.

Proposed Course of Project: Further studies are planned to attempt elucidation of the mechanism(s) of polysome breakdown during the period of hypoglycemia and their subsequent restoration during the recovery. Using radioactive metabolic precurses of the nucleic acids and protein, alteration of rates of synthesis of nucleic acids and protein during the hypoglycemia and subsequent restoration period will be studied.

Publications: None

Serial No. NDS(I) 69-LPP 1711
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Callosal localization of transfer of tactuokinesthetic information in the rhesus monkey

Previous Serial Number: None

Principal Investigator: Dr. Gilbert A. Preston

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives: To localize that area of the corpus callosum responsible for intermanual transfer of a learned motor skill.

Methods Employed: A series of animals with graded transections of the anterior and posterior portion of the corpus callosum were prepared. They were trained and tested for intermanual transfer of a latchbox skill with no visual access to the task. The approximate borders for this functional sector appear to be anteriorly about 10 mm from tip of genu and posteriorly about 8-10 mm anterior to tip of the splenium.

Significance: The data confirms the rold of the forebrain commissures in intermanual transfer of a motor skill and reveals an unexpected equivalence for those portions of the commissure relating to precentralgyrus and to parietal lobe in this function.

Publications: None

Serial No. NDS(I) 69-LPP 1712
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Barbiturate effects on brain glycogen in the monkey

Previous Serial Number: None

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Ronald E. Myers

Cooperating Units: None

Man Years:

Total:	0.1
Professional:	0.1
Others	0

Project Description:

Objectives: The effects of barbiturate anesthesia on brain glycogen is studied.

Methods Employed: The monkey is deeply anesthetized with barbiturate and respired artificially to maintain the blood gases and the acid-base status at normal levels. Different animals will be sacrificed after 3, 6, 12, 24, 48, and 92 hours of anesthesia. The brain and other tissues will be removed, frozen and assayed for glycogen levels.

Major Findings: After 24 hours of deep barbiturate anesthesia there is a three-fold increase in glycogen.

Significance: To learn if depression of neural function affects the levels of glycogen in the brain. The answer to this question may help explain the transitory increase in glycogen seen after acute total asphyxia.

Proposed Course of Project: To continue project as described.

Publications: None.

Serial No. NDS(I) 69-LPP 1713

1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Glycogen levels in the brain of the recovering hypoglycemic monkey

Previous Serial Number: None

Principal Investigator: Dr. Americo Rivera, Jr.

Other Investigators: Dr. Kenneth Kahn

Cooperating Units: None

Man Years:

Total:	.5
Professional	.2
Other	.3

Project Description:

Objectives: The glycogen levels of the brain and other tissues of the juvenile monkey recovering from an episode of prolonged hypoglycemia is under investigation.

Methods Employed: Juvenile monkeys are subjected to 6 hours of hypoglycemia. The animals are sacrificed at various time intervals after the insult. The animals are anesthetized and the brain, heart, lung, liver, kidney, spleen, and muscle quickly excised and frozen in a mixture of isopentane and isohexane cooled with liquid nitrogen.

Major Findings: During hypoglycemia the glycogen levels of the brain are markedly depleted. However, by twelve hours after the insult the brain glycogen is approximately twice the normal content and remains high for at least 60 hours. Animals are presently being prepared for long-term study to determine when the brain glycogen levels return to normal.

Significance: This study should yield insight into some biochemical problems affecting the survival of subjects suffering a prolonged period of hypoglycemia (insulin shock).

Proposed Course of the Project: To obtain sufficient animals to outline the levels of glycogen in the brain at various times after the insult and to determine the time required to return to normal.

Publications: None.

Serial No. NDS(I) 69-LPP 1714
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Blood/Cerebrospinal fluid relationship in acute asphyxia

Previous Serial Number: None

Principal Investigator: Dr. Harvey M. Shapiro

Other Investigators: Dr. Ronald DiGiacomo

Cooperating Units: None

Man Years:
Total: .3
Professional: .3
Other: .0

Project Description:

Objectives: Determination of arterial blood/cerebrospinal fluid relationships during acute asphyxia in the adult monkey and correlation of this data with last gasp phenomena and similar information presently available on the monkey neonate.

Methods Employed: Adult rhesus monkeys previously used in physiological psychological studies (operated and unoperated) and in studies on hypoxia (fully recovered) are anesthetized with intravenous barbiturate and subjected to three asphyxial protocols: 1) Tracheal clamping; 2) High-flow 100% Nitrogen; 3) Hyperventilation with 100% Nitrogen. The abdominal aortic blood pressure, respiratory gasps, arterial and cerebrospinal fluid blood gas tensions and acid-base status are monitored.

Major Findings: Cardiovascular collapse (mean BP below 50) occurs from 5 - 8' in all 3 protocols. Hyperventilation abolishes the first gasping activity which occurs under nitrogen inhalation. A second phase of gasping occurs shortly after the mean BP has fallen below 50mm Hg in all 3 groups. Metabolic and respiratory acidosis are reflected in both arterial blood and the CSF. Hyperventilation results in an asphyxial death with an alkaline arterial blood. The trend toward increased CSF acidity is only moderately attenuated by hyperventilation.

Significance: The cerebrospinal fluid pool rapidly reflects acute asphyxial changes in the rhesus monkey. These changes still occur with alkaline arterial blood.

Proposed Course of Project: Several more experiments will be carried out to provide 10 animals in each of the three protocol groupings.

Publications: None.

Serial No. NDS(I) 69-LPP 1715
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Effect of hypoxic hypoxia on the monkey brain
(Title changed from: Metabolic factors involved in the production of hypoxic cerebral edema)

Previous Serial Number: None and incorporating Serial No. NDS(I) 68-LPP 1564

Principal Investigator: Dr. Harvey M. Shapiro

Other Investigators: Dr. Ronald E. Myers
Dr. Richard Hibbs
Dr. Robert Petersen

Cooperating Units: Laboratory of Molecular Biology, NINDS, NIH

Man Years:
Total: .2
Professional: .2
Other: .0

Project Description:

Objectives: The hypoxic hypoxia model is developed for study of brain changes associated with hypoxia in the absence of arterial hypotension or circulatory stasis. This preparation isolates the effect of oxygen deprivation upon brain chemistry, acid-base status, electrical activity, and morphology.

Methods Employed: Under intravenous barbiturate anesthesia the adult monkey is exposed to a 2.5 - 3.5% mixture of oxygen in nitrogen administered by a mechanical ventilator which is adjusted to yield a mild hypocapnia.

Cerebrospinal fluid and blood gas tensions and acid-base status are monitored. Small amounts of cardiotonic drugs are administered to permit adjustment of the arterial blood pressure. The jugular venous pressure, electroencephalogram, brain electrical impedance, electrocardiogram, and at times the intracranial pressure are monitored.

Animals for optical microscopy survive for a period of three weeks and are then formalin-perfused and their brains examined grossly and histologically. Animals for electronmicroscopy are perfused with glutaraldehyde at different stages of insult and recovery. Luciferene assays for ATP levels are performed on animals sacrificed at various times during hypoxia and recovery.

Major Findings: Hypoxia without vascular hypotension results in an isoelectric EEG in 3-8 minutes in the present experimental procedure utilizing 2.5 - 3.5% oxygen mixtures. The arterial pO_2 is 18 and the jugular venous pO_2 is 12. Both arterial blood and cerebrospinal fluid develop marked acidosis. There is a significant decrease in CSF bicarbonate. Administration of 100% oxygen after the insult period results in a jugular venous blood hyperoxia as manifested by a pO_2 range of 275-310. The duration of the jugular venous hyperoxia appears to relate to the duration of the hypoxia, when arterial blood pressure is maintained over a mean of 80mm Hg. Below mean arterial pressures of 65, the degree of hyperoxia is enhanced and the hyperoxic period prolonged. This result indicates a greater effect upon the brain of the standard hypoxic insult at lower arterial perfusion pressures.

The hypoxic insult results in a significant systemic and cerebrospinal fluid acidoses. Animals with the higher range of perfusion pressures have lesions limited to the globus pallidus. Biochemical analysis reveals a tenfold decrease in cortical ATP soon after the onset of 3.5% O_2 administration. Brain electrical impedance increases 20-30% with an onset latency of 2-4 minutes. The impedance returns toward its baseline values rapidly after the administration of 100% oxygen if there has been no hypotension.

Significance: Remarkable degrees of resistance of the brain to profound hypoxia are demonstrated if the blood pressure is maintained above a mean of 65mm Hg. Prolonged jugular venous hyperoxia is a sign of a severe insult to the brain. The recovery slope of the J.V. hyperoxic phase may have prognostic value with clinical applications.

Proposed Course of the Project: Establishment of the hypoxic hypoxia model will allow further studies (EM, Biochemical) of brain energy deprivation states.

Publications: None

Serial No. NDS(I) 69-LPP 1716
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental placental insufficiency in sheep

Previous Serial Number: None

Principal Investigator: Dr. Harvey M. Shapiro

Other Investigators: Dr. Ronald E. Myers
Dr. Donald Cheek

Cooperating Units: Dairy Cattle Research Branch
U. S. D. A., Beltsville, Maryland

Department of Pediatrics, Johns Hopkins Hospital
Baltimore, Maryland

Man Years:
Total: .2
Professional: .2
Other: .0

Project Description:

Objectives: 1) Effects of surgical removal of placental mass at two gestational ages upon the fetus. 2) Effects of partial placental extirpation upon placental growth and/or regeneration.

Methods Employed: Laparotomies are performed upon ewes with dated pregnancies, under barbiturate anesthesia and mechanical respiration for amputation of the distal tip of the gravid horn at 50 and 90 days of gestation. With this method partial removals of placenta are possible leaving intact the ovary and vascular plexus of the uterus. Sham operations are performed in a similar fashion but confining the horn amputation to the distal 2-3cm tip of the horn and thereby removing no placenta. Non-operative control animals are also included in the study and type of operation (sham vs operative) and amount of placental tissue removed is randomized. One hundred and forty ewes are included in the study. At 140 days of gestation (normal gestation = 150) the lambs are delivered by caesarean section and bodyweight, body length, and organ weights determined. Cord blood and tissue aliquots are immediately frozen and submitted to various biochemical analytical procedures.

Major Findings: 1) Partial removal of placenta results in subtle changes in fetal weights and organ weight ratios which are revealed by the application of the discriminant function test. Brain weight is little affected while liver weight appears to vary with the amount of placenta present at term. Study of organ weight ratios reveals no significant differences. 2) Histological analysis reveals no striking changes. 3) Biochemical analyses have revealed a decrease in the cell population in the brain although it achieves normal weight ranges. The total non-collagen protein content of the placenta relates proportionally to the weight of the liver. There is a decrease in RNA content of the cerebellum. Myocardial hypertrophy (increased cell size) occurs in the absence of changes in skeletal muscle. 4) Operative abortion rates at 50 days are 15% and approach 60% when operative intervention is performed at 90 days of gestation.

Significance: This study relates to the problem of dysmaturity or fetal growth retardation and its conditions of occurrence.

Proposed Course of Project: Delivery of remaining operative, sham and control animals. Submission of data to the discriminant function test to confirm statistical formulas developed to discriminate the occurrence of placental insufficiency.

Publications: None

Serial No. NDS(I) 69-LPP 1717
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Organization of cortico-cortical projection.

Previous Serial Number: None

Principal Investigator: Dr. William D. Thompson

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.2
Professional: 1.0
Other: 0.2

Project Description:

Objectives: There is abundant evidence of the anatomical interconnections between related cortical regions. However, little is known of the basic organization of these connections or of the information transfer over these connections. An attempt has previously been made to establish an input-output relationship for minimal organizational units of primary sensory cortex in cats. This work is being extended in primates in which the organization of the different regions of sensorimotor cortex is more clearly appreciated.

Methods Employed: The utilization of intracortical microstimulation wherein small amplitude current pulses are passed through microelectrodes, allows limiting direct electrical excitation to small groups of functionally related neurons. By utilizing the same microelectrode for both recording and stimulation, the output from vertical columns of sensory cortex with defined peripheral input onto cells of motor cortex can then be determined. Comparison of the peripheral inputs to the two interrelated regions establishes an input-output relation for that column of sensory cortex.

Major Findings: This project has just been initiated and no concrete data has yet accrued from work on primates. Previous work on cats suggests that information regarding a specific peripheral locus is forwarded from highly restricted foci in sensory cortex into a discrete region of motor cortex. That this is not the sole sensory pathway into motor cortex is attested to by the fact that abolishing activity in sensory cortex by cooling does not block

peripheral activation of cells in motor cortex.

Significance: The tactile placing reaction can be abolished by lesions of either precentral or postcentral gyrus, suggesting that the postcentral gyrus acts as the input component and the precentral gyrus as the output component for this reaction. Examination of the minimal organizational units for this complex motor activity should be enlightening with regard to the interactions of different cortical regions.

Proposed Course of the Project: To be continued as proposed.

Publications: None

Serial No. NDS(I) 69-LPP 1718
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Social organization and behavior of a small band of free-ranging rhesus monkeys.

Previous Serial Number: None

Principal Investigator: Dr. Margaret Varley

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .7
Professional: .7
Other: .0

Project Description:

Objectives: To describe the social organization of a small band (31 monkeys) of rhesus monkeys, in terms of its dominance structure and major affinities or associative bonds. To describe the development of the infant monkey from day 1 to six months in terms of its social communication and contacts with same and other age monkeys.

Methods Employed: Regular observation of from 15 to 25 hours per week is made of a small band (Group J) of monkeys on Cayo Santiago. Sociometric measures of the participants of play, mating, close physical proximity, grooming and fighting are recorded. Observation is also being made of interactions between infants and mothers, and between infants and other monkeys within the troop. Particular emphasis is placed on the development of aggressive and submissive gestures in the infant. Some of these interactions are being recorded on 16 mm movie film for detailed analysis.

The major attempt will be to define as precisely as possible the type of social contact, including play, rather than to quantify shifts in overall amount of contact through time, which has already been done in the laboratory and in the free-ranging situation.

As time permits an alternate group of monkeys (Group I) of similar size but of different composition is being observed to provide a contrasting group and a wider range of maternal and infant behaviors.

Major Findings: The analysis of sociometric data is not complete. Overall structure is compatible with the description of another group (F) on this island, with certain qualifications. Birth season has just begun in this group (two infants) and movie analysis is in its beginning phases.

Significance: Structure of the social organization of a small group of rhesus monkeys as described by Sade (for Group F) needs verification and comparison with other groups. Although descriptions have been made of mother-infant and infant-infant interactions, precise definition is lacking. This definition, if pursued over a number of years, may shed light on discrimination between the effect of experience with peers versus experience with the mother and other adults, and to an analysis of aggressive and appeasement gestures, in the sense of their progression from full expression to components or from components to full expression.

Proposed Course of the Project: To carry out the study as planned.

Publications: None

Serial No. NDS(I) 69-LPP 1719
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Adaptation and seasonality of a small group of rhesus monkeys in a new environment.

Previous Serial Number: None

Principal Investigator: Dr. Margaret Varley

Other Investigators: Dr. Stephen Vessey
Dr. Halsey M. Marsden

Cooperating Units: None

Man Years:
Total: .10
Professional: .05
Other: .05

Project Description:

Objectives: Two families of free-ranging rhesus monkeys have been transferred from one island facility to another. These monkeys are being observed: (1) to describe their adjustment both to the new situation and to the four bands of free-ranging rhesus monkeys already on the island, (2) to determine the onset of seasonality of their mating behavior in a situation where environmental conditions lead to a breeding season three months later than in the former situation.

Methods Employed: Members of two geneologies from Group C on Cayo Santiago were transported and retained in cages at the field station at La Parguera during the months of December, January and February. They were released to the free-ranging conditions on Cueva Island early in March, 1969. The group is made up of 30 monkeys, 22 females and six males from the two families, and two additional adult males from Group C.

The monkeys are being observed in their overall adaptation to this new environment, particularly in their ability either to form one or more bands among themselves or to join existing bands on the island. Data will be recorded as well on the occurrence of mating behavior and date of births of subsequent offspring. Comparisons will be made between monkeys differing in their reproductive statuses: ten gave birth in 1968; eleven were presumably in estrus in the fall of 1968, and of these some will give birth and others

will not. Five approach their first breeding season. Comparisons among the males from Cayo Santiago and those from La Cueva, some of whom may cross groups, may give additional information.

Major Findings: This study is in its initial stages.

Significance: This is the first investigation of adaptive behavior among a group of related monkeys whose past history is known, when they must adjust to a new environment with pre-existing bands of the same species monkeys in the free-ranging situation.

Breeding season of the two situations - the new and the old - differs by three months and the onset has been positively correlated with onset of heavier rainfall. After eight months of living in the new situation, the monkeys may respond principally in terms of environmental conditions with breeding occurring during the later season (November through February). It is possible that a longer ranging (internal) physiological cyclicity may result in a breeding season which is earlier (August through December). Comparisons among classes of females and of males may give more information about the physical, social, and physiological determinants of mating behavior.

Proposed Course of the Project: To carry out the project as planned.

Publications: None

Serial No. NDS(I) 69-LPP 1720
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Early vs. late forebrain commissure section on interocular transfer of visual learning.

Previous Serial Number: None

Principal Investigator: Dr. Shun-ichi Yamaguchi

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: To investigate whether forebrain commissure section during the newborn period produces different effects on interocular transfer of visual learning compared with the section in later life.

Methods Employed: Four monkeys who had received midline section of the corpus callosum and the anterior commissure within the first week after birth, and four other monkeys who received the same surgery at approximately two years of age underwent section of optic chiasm at 3 to 4 years of age. They were trained on several varieties of visual discrimination tasks using only one eye and the contralateral arm. After each task was learned, the eye-hand restriction was switched to opposite members and interocular transfer of learning tested.

Major Findings: Both groups failed to show interocular transfer of visual discrimination learning with no differences showing up between the two groups.

Significance: Unlike lesions of cortex produced in early life, transection of corpus callosum at the time of birth produces the same effects as a comparable lesion in later life.

Proposed Course of the Project: Project completed.

Publications: None

Serial No. NDS(I) 69-LPP 1721
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Cerebrospinal fluid composition during development.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Laszlo Bito

Cooperating Units: Department of Ophthalmology, Columbia University, School of Medicine, New York

Project Description:

Objectives: To determine the alterations in composition of cerebrospinal fluid during in utero fetal development and during maturation into adult life. Particular attention is paid to magnesium, potassium, and calcium.

Methods Employed: For that part of the study involving fetal animals the pregnant female is anesthetized with pentobarbital injections. The fetus is delivered by cesarean section. Samples of cerebrospinal fluid are taken from the lateral ventricle, the cisterna magna, the subarachnoid spaces over the convexities and from the lumbar sac. In addition, samples of blood are taken. Juveniles and adults,of known ages, are also anesthetized and samples of cerebrospinal fluid in the different locations obtained to determine possible alterations in composition associated with maturation beyond birth.

Major Findings: In the adult the potassium levels of the cerebrospinal fluid in the different locations are maintained and actively regulated at levels significantly below (-30%) that of an ultrafiltrate of plasma. As the fluid passes from its site of origin in the lateral ventricles to its site of absorption over the convexities, there is a progressive decrease in concentration of potassium indicating further potassium loss from the cerebrospinal fluid into the nervous system parenchyma through the pia-glial limiting

membrane. This is presumed to be due to further active pumping of potassium from the cerebral substance by the blood brain barrier into the cerebral blood vessels. By sampling fetuses and monkeys of different ages it has become clear that the regulation of potassium in the cerebrospinal fluid at adult levels develops prior to birth at approximately the 100th gestational day. Prior to this time the levels of potassium are comparable to those of a plasma ultrafiltrate. Cerebrospinal fluid magnesium levels, in contrast, are regulated at a much higher concentration in the cerebrospinal fluid than in the plasma (+50-60%). Curiously, the adult levels of magnesium are reached early in development even prior to 40 gestational days. Calcium levels are more variable but are regulated downward by the choroid plexus during its formation and are maintained at levels approximating one-half of that of its concentration in the plasma.

Significance: It is hoped as the time of beginning regulation of different constituents in the cerebrospinal fluid is more precisely determined that the initiation of such regulation may be related to manifestations of nervous system activity or alteration in cellular functions.

Proposed Course of Project: Several gaps are being filled in. The project will be completed during the course of this year.

Publications: None.

Serial No. NDS(I) 69-LPP 1722
1. Intramural Research
2. Laboratory of Perinatal Physiology
3. San Juan, Puerto Rico

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Validity of fetal scalp sampling as indicator of fetal state during labor and delivery.

Previous Serial Number: None

Principal Investigator: Dr. Ronald E. Myers

Other Investigators: Dr. Karlis Adamsons
Dr. Richard Beard

Cooperating Units: Department of Obstetrics and Gynecology
Columbia University School of Medicine
New York and
Queens Charlotte Hospital, London, England

Project Description:

Objectives: To determine the validity of the fetal scalp sample as taken during labor or delivery as indicator of fetal circumstance.

Methods Employed: Term monkey fetuses are delivered by cesarean section and left attached to an intact placenta by the umbilical circulation. Major vessels are catheterized including the carotid artery on the right and the jugular vein on the left, electrocardiographic leads are placed across the chest, and a catheter placed in the uterus for recording of intrauterine pressures. The fetus is restored to the uterus and the uterus and maternal abdominal wall sutured closed. The physiological status of the fetus is monitored in addition to the acid-base and blood gas status of blood samples withdrawn from the major vessels. Prior to fetal return to the uterus, the internal cervical os is dilated manually and the fetal head replaced as a presenting part. Labor is induced by an I.V. drip of oxytocic agents. At periodic intervals during such artificially induced labor, samples of fetal scalp blood and of blood from the major vessels are withdrawn simultaneously. Fetal scalp samples are taken using a vaginal speculum and a small blade producing prick incisions in the presenting scalp. Comparison of the acid-base and blood gas status of the samples taken from the two sites yield clear-cut indication as to the

validity of the fetal scalp sample as a reliable indicator of fetal status.

Major Findings: The values of the fetal scalp samples consistently lay between the similar values obtained from the carotid artery and the jugular vein. Thus, the fetal scalp sample appears to be a reliable indicator of fetal circumstance during labor.

Significance: It is of great significance to develop techniques for evaluation of the fetal status during labor and delivery, particularly in complicated cases. In more recent times it has been found that the fetal heart rate is an entirely inadequate indicator of fetal status. The recently developed techniques for fetal scalp sampling studied in the present circumstance present the opportunity for a rapid, relatively safe, and reliable indicator of fetal status.

Proposed Course of Project: Project completed.

Publications:

Adamsons, K., Beard, R. W., Cosmi, E. V., and Myers, R. E.: The validity of capillary blood in the assessment of the acid-base state of the fetus. In: Diagnosis and Treatment of Fetal Disorders. Ed. by K. Adamsons. Springer-Verlag, New York, 1968.

Adamsons, K., Beard, R. W., Myers, R. E.: Comparison of the composition of arterial, venous and capillary blood of the fetal monkey during labor. Amer. J. of Obstet. & Gynec., (In Press) 1969.

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